IRANIAN JOURNAL of IMMUNOLOGY

Volume 20, Supplementary Issue, April 2023
16th International Congress of Immunology and Allergy

www.iji.ir

Allergy and Immunotherapy

Allergy co-factor existence prediction using bipartite graph modularity applied to co-occurrence matrix

Fatemeh Kaveh-Yazdy[1]

1. *Yazd University of Medical Sciences, Yazd, Iran*

Background: Predicting allergies plays a critical role in avoiding allergic reactions. While identical/comparable reactions. The aim of this article is to adopt machine learning methods to predict the likelihood of co-factors among allergies with respect to their co-occurrences.

Methods: In this research, we utilize allergens that are different, they may have common components or similar structures leading to the data collected through a cross-sectional cohort of 333200 children in Philadelphia. Data includes the occurrence history of food and non-food allergies in human subjects. We apply the bipartite graph modularity to extract the allergies that are likely to co-occur. Consequently, co-occurred allergies might have similar molecular/environmental co-factors. In this study, data are randomly partitioned into two separate subsets, a.k.a., training set, and test, t set, and results are evaluated on the test set using the model trained on the training set. The number of groups, in which allergies are scattered based on their co-occurrences is determined using eigengap analysis.

Results: our method predicts the occurrence of an allergy in non-adults human subjects with respect to their history of allergies. To evaluate our findings, we use mathematical analysis to show that the best number of clusters is found. Furthermore, our results resemble the co-occurrence ratio of allergies in similar studies, e.g. the estimated likelihood of simultaneous allergy to walnut and peanut is widely reported in allergy epidemiological analysis. Findings showed shellfish allergy has the lowest confidence as a prior allergy to predict other allergies.

Conclusion: With respect to the previous findings, allergies are not randomly co-occurred, and they share similarities in structure/environment factors. In this way, we propose a machine learning method to take into account the co-occurrences of allergies to predict the likelihood of happening unseen allergies. Due to few numbers of allergies, this method is a tolerably low-cost way of predicting allergies to prevent severe outcomes.

Keywords: Food Allergy, Graph Modularity, Co-Clustering, Eigen-gap Analysis.

Anti-Inflammatory Effect of Bevacizumab in Rat Model of Asthma

Seyed mohammadreza Bolandi[1], Zohreh Abdolmaleki[2], Mohammad-Ali Assarehzadegan[3], Erfan Mohammadi Sepahvand[4]

1. Department of Pharmacology, Karaj Branch, Islamic Azad University, Karaj, Iran
2. Department of Pharmacology, Karaj Branch, Islamic Azad University, Karaj, Iran
3. Immunology research center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
4. Department of Microbiology and Immunology, Karaj Branch, Islamic Azad University, Karaj, Iran

Background: Inflammation, airflow obstruction, elevated bronchial blood pressure, and external conditions all play a role in the highly prevalent condition known as asthma. The most significant aspects of clinical symptoms are the contraction of smooth muscles and inflammation, which cause the airways to constrict and become obstructed. Numerous stimuli can cause bronchial blockage, including respiratory infections, allergic reactions, irritants, exercise, and non-steroidal anti-inflammatory medicines. Bevacizumab is a humanized anti-VEGF monoclonal antibody that inhibits angiogenesis and it is used to treat several malignancies.

Methods: Twenty-one male Wistar rats were placed into three groups, each with seven animals, at random: control, ovalbumin (OVA)-sensitized, and OVA+Bmab (OVA+Bmab). OVA and OVA+Bmab groups were exposed to ovalbumin (OVA) and aluminum hydroxide for sensitization on days 1, 8, and 15 before being challenged with OVA atomized for ten days (inhalation). Bevacizumab was given to the OVA+Bmab group after OVA sensitization for two weeks. Genes and protein expression of several anti-inflammatory and pro-inflammatory cytokines were assessed and VEGFR2 was semi-quantitatively analyzed in the lungs by immunohistochemistry. In this study, also goblet cell population, the inflammatory cell population in bronchial alveolar lavage fluid (BALF), and the level of OVA-Specific IgE in serum blood samples were evaluated.

Results: Bevacizumab effectively reduces bronchial inflammation by reducing VEGFR2 expression, followed by a decrease in inflammatory cytokines and an increase in the release of anti-inflammatory cytokines ($p \leq 0.05$). Furthermore, the OVA-specific-IgE level in the OVA+Bmab group significantly decreased. Moreover, the number of macrophages, neutrophils, and lymphocytes in the OVA+Bmab group significantly reduced compared to the OVA group ($p \leq 0.05$).

Conclusion: According to the findings, VEGF has a vital role in the production of inflammatory mediators in lung airways in asthma. Targeting VEGF through the application of anti-VEGF agents such as Bevacizumab would be a great therapeutic approach to improve asthma.

Keywords: Bevacizumab, Asthma, Inflammation, VEGF

Association between the IL-7Rα gene polymorphisms of rs6897932 and rs987106 with atopic diseases

Nahid Eskandari[1]

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Atopy is used to describe the genetic tendency to IgE-mediated responses. Many factors contribute to predisposing individuals to atopy, including cytokines. IL-7 is a cytokine that mediates hypersensitivity responses and proliferation of T cells and has a polymorphic gene for its receptor (CD127). These different alleles may affect the signaling cascade or alter the production of inflammatory cytokines, which may exacerbate atopic conditions, including asthma and allergy. Primary objective of our study aimed to explore the linkage between IL-7Rα gene polymorphism at the SNP of rs6897932 and the risk of atopic diseases. Evaluating the association of-7RαSNP ofrs987106 and also serum levels of IL-7 with the risk of atopy are among the secondary objectives.

Methods: To seek possible relations of SNPs rs6897932 and rs987106 with the risk of atopy, we recruited 101 atopic patients and 201 sex-age-matched healthy controls from Iranian ethnicity and used the data for evaluation.

Results: Statistical analysis revealed that the T allele variant of the IL-7Rα gene at the SNP of rs6897932 is more prevalent among the atopic patients (53.5%) than in the controls (26.9%) and has a considerable association with the disease ($p< 0.0001$, OR: 3.13 (95% CI- 1.90 to 5.16)). Also, the higher frequency of the T allele variant of rs987106 in atopic patients (64.4%) versus control (31.3%) was associated with around a 4-fold increase in the risk of atopy ($p <0.0001$, OR: 3.95 (95% CI- 2.39 to 6.55). However, there was no significant association between IL-7 serum levels and atopic disorders ($p > 0.05$).

Conclusion: Our study in the Iranian population may support that T allele variants of rs6897932 and rs987106 are risk factors for atopy.

Keywords: IL-7R, gene, polymorphisms, atopic

Characterization of allergenic proteins in five common regional plant pollens

Sajjad Chamani[1]

1. Birjand University of medical science, Birjand, Iran

Background: Allergic disorders are common health problems with a negative impact on patients' quality of life and high economic burden. Pollen grains are the main trigger of allergic symptoms in most parts of the world. Knowing the main source of allergenic pollens in each area is the first step in the prevention and management of inhalant allergic disorders. This study aimed to evaluate the allergenicity and immunochemical characterization of five common regional plant pollens.

Methods: Pure pollen of five abundant plants in the area, including the Eucalyptus tree (blue gum), Rosa damascene flowers (Damask rose), Jasminum sp flowers (Persian Jasmine), Phoenix dactylifera tree (palm), and Pistacia vera trees (pistachio), were collected during pollination season and then glycerinated aqueous extracts were prepared., Skin prick test with prepared extracts, as well as commercial extracts, was performed on thirty-seven patients with a history of allergic rhinitis. The protein profile of domestic extracts was evaluated by SDS-PAGE and, the allergenic profile was investigated by immunoblotting using pooled serum collected from sensitive patients.

Results: The highest and lowest prevalence of skin sensitivity to domestic extracts was for pollens of the Eucalyptus tree (59.45%) and the Phoenix dactylifera tree. The largest mean wheal size was for Rosa damascene flowers and, the Eucalyptus tree. The resolved protein fractions on SDS-PAGE ranged from 5–85 kDa. In western blotting, all extracts had several reactive bands but the major reactive bands for each extract were as follows: Eucalyptus 55 kDa, Jasminum sp 45 kDa, Phoenix dactylifera 40 kDa, Rosa damascene 30 kDa and, Pistacia Vera 25 kDa.

Conclusion: The result of this study showed that selected pollens have several allergens which can induce hypersensitivity reactions.

Keywords: Allergens, Allergic rhinitis, IgE, Immunoblotting, Pollen, SDS-PAGE

Effect of calcitriol-treated mesenchymal stem cells as an immunomodulation micro-environment on allergic asthma in a mouse model

Majdedin Ghalavand[1], Samira Mohammadi-Yeganeh[2], Seyed Mahmoud Hashemi[3], Hadi Esmaeili Gouvarchin Ghale[4]

1. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Medical Nanotechnology and Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: Allergic Asthma is a chronic inflammatory illness of the respiratory system characterized by an increase in the number of inflammatory cells in the airways and trouble breathing. Mesenchymal stem cells (MSCs) have the potential to be used in inflammatory diseases as a cellular immunosuppressive treatment. They express calcitriol receptors and communicate with other immunocytes, which increases their anti-inflammatory activity. This study aimed to determine the effects of calcitriol-treated MSC treatment on allergic asthma pathways in a mouse model.

Methods: To generate a mouse model of asthma, the mice were sensitized intraperitoneally with ovalbumin (OVA) and aluminum hydroxide emulsion and then challenged intra-nasally with OVA. On day 14, experimental mice received tail vein injections of calcitriol-treated MSCs in PBS prior to allergen exposure. The cytokines assay including IL-4, 10, 12, 17, TGF-β and IFN-γ, splenocytes proliferation, and histological examination of lung samples were performed. The mice were sensitized with OVA and the response to dexamethasone treatment was compared.

Results: Calcitriol-treated MSCs significantly increased levels of IL-12, TGF-β, and IFN-γ compared to non-treated MSCs groups. Moreover, calcitriol-treated and non-treated MSCs significantly decreased IL-4 and 17 compared to asthmatic groups. The results of the histopathological examination showed that calcitriol-treated MSCs reduced the accumulation of inflammatory cells and bronchial wall thickening in comparison with the asthma group.

Conclusions: Using the allergic asthma model, we were able to show that calcitriol-treated MSCs had an inhibitory impact on airway inflammation. Our findings suggest that the injection of calcitriol-treated MSCs may be a viable treatment option for allergic asthma.

Keywords: Experimental asthma, Calcitriol, Mesenchymal stem cells

Epicutaneous immunotherapy using DC-targeted gold nanoparticles as an efficient carrier in Mice Sensitized to pollen (Platanus orientalis)

Safoora pordel[1], Mojtaba Sankian[2]

1. Immunology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Immunology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Epicutaneous immunotherapy (EPIT) is a novel allergen immunotherapy (AIT) for allergen-specific allergic diseases. Various studies have shown EPIT is as efficacious as subcutaneous immunotherapy (SCIT), which is considered a reference immunotherapy. It has also been shown that targeted allergen delivery systems and skin-penetrating peptides (SPPs) can increase EPTT efficacy. In this study, we used functionalized gold nanoparticles (GNPs) along with the DC-specific aptamers as the targeted allergen delivery system aimed to EPIT with Platanus orientalis pollen protein extract in rhinitis allergic mouse models. Moreover, SPPs were also used to enhance intact skin permeability and improve EPTT efficacy.

Methods: BALB/c mice were sensitized by the subcutaneous route to Platanus orientalis pollen protein extract, treated for 8 weeks using EPIT in different groups with the free pollen protein extract, different doses of nanogold- DC-specific aptamer complexes and nanogold with the total pollen protein extract. Measurements involved the serological response (total and specific IgE) and cytokine profile (IL-4 and IFN-γ), of splenocytes reactivated with the pollen protein extract and recombinant protein allergens of Platanus orientalis pollen and splenocyte proliferation by MTT. In addition, the total cell count and differential eosinophil count of nasal lavage fluid (NALF) were calculated.

Results: Our study results revealed that EPIT in GNPs and aptamer-treated groups had a significant increase in IFN-γ and a significant decrease in IgE and IL-4 concentrations, as well as NALF, infiltrated immune cell count, and eosinophil counts compared to the non-targeted groups.

Conclusions: Our findings indicate that GNPs and targeted-GNPs with DC-specific aptamers could act as an efficient approach for the improvement of EPIT efficacy compared to free pollen protein extract mixed.

Keywords: allergen immunotherapy, Epicutaneous immunotherapy, gold nanoparticles, aptamers

Identification of encoding genes of ns-LTP proteins in Solanum melongena plant and prediction of major allergenic epitopes of these proteins by immunoinformatic tools

Sajad Azimi Mehrabadi[1], Mohammad-Hossein Shams[1], Ali Sheikhian[1], Seyyed Mohsen Sohrabi[2], Mohammad-Ali Assarehzadegan[3], Ali-Mohammad Varzi[1]

1. Department of Medical Immunology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.

2. Department of Production Engineering and Plant Genetics, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Immunology Research Center, Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Today, food allergies have become a major problem all over the world, especially in developed countries. These allergies are increasing. As an edible plant scientifically called Solanum melongena, eggplant has been reported to cause allergies in many different communities that have this plant in their diets. The mechanisms and molecules that are probably involved in causing allergic reactions to Solanum melongena include ns-LTP proteins, profilins, PPOs, histamine, tyramine, tryptamine, alkaloids, and phytoestrogens. The aim of the present study was to identify the genes encoding ns-LTP proteins in the Solanum melongena plant through the polymerize chain reaction (PCR) method, and then determine the physicochemical characteristics and identify the main allergenic epitopes in these proteins using immunoinformatic methods.

Methods: In this study, first the presence of ns-LTP genes in S. melongena was investigated using the PCR method and after sequencing these genes, the assembled sequences were aligned against the GenBank nr database using the BLASTn tool. Then, the amino acid sequences of ns-LTP proteins were analyzed through bioinformatics and immunoinformatic tools.

Results: In this study, it was found that there are 21 genes encoding ns-LTP proteins in the Solanum melongena plant. Two major allergenic epitopes of ns-LTP-8 protein were predicted to bind to B lymphocytes with higher affinity binding more than 0.6. nsLTP8 and nsLTP10 had the most allergenic epitopes with high binding affinity to B and T lymphocytes, respectively.

Conclusion: In the present study, through immunoinformatic tools, it was predicted that ns-LTP-8 and ns-LTP-10 proteins would have the most epitopes with potential allergenicity properties in S. melongena. Probably, these proteins have the most important role in food allergy to ns-LTP proteins in S. melongena.

Keywords: Solanum melongena, Allergy, Allergen, nsLTP

Immunochemical Characterization of Salix alba Pollen Allergens

Ali Bagersad[1], Mohammad-Hossein Shams[1], Fatemeh Omidi[1], Farhad Shahsavar[1], Ali-Mohammad Varzi[1], Mohammad-Ali Assarehzadegan[2], Seyyed Mohsen Sohrabi[3]

1. Department of Medical Immunology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.

2. Immunology Research Center, Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Production Engineering and Plant Genetics, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Background: One of the most common causes of allergic respiratory diseases is the existence of pollen grains in the air, especially allergenic trees and plant pollens. One of the sources of these allergenic pollens is Salix trees, which belong to the Malpighiales order and Salicaceae family. Allergy to pollens of different species of Salix trees has been reported in different parts of the world. The most common type of Salix tree in Iran is white willow (Salix alba).

Objectives: This study aimed to identify and determine the immunochemical characteristics of IgE-binding proteins in S. alba tree pollen extract using SDS-PAGE and IgE-immunoblotting methods.

Methods: Using the skin prick test, 20 patients with clinical symptoms of allergic rhinitis were included in the study. Through SDS-PAGE and IgE-immunoblotting methods, the reaction of allergenic proteins in S. alba pollen extract with specific IgE antibodies in patients' sera was investigated.

Results: SDS-PAGE in reducing conditions showed that there are more than 17 protein bands in S. alba tree pollen extract, whereas, in non-reducing conditions, the number of protein bands was less. Using the sera of the studied patients, more than 11 protein bands binding to specific IgE antibodies with a molecular weight of approximately between 13 and 95 kDa were identified in S. alba tree pollen extract. The results of immunoblotting showed that proteins with a molecular weight of approximately 15 (85%), 25 (40%), 40 (40%), and 60 (45%) kDa have the highest reaction with the IgE antibody of patients' serum.

Conclusion: In this study, it was found that more than 80% of the sensitive patients who participated in the study had specific IgE antibodies reacting with the approximately 15 kDa protein present in S. alba pollen extract. For this reason, the 15 kDa protein was considered the main allergen of S. alba tree pollen.

Keywords: Salix alba, pollen, allergy, allergen

Increased TGF-β secretion and reduced eosinophil infiltration following prophylactic sublingual regimen using ovalbumin-enriched mesenchymal stem cell-derived exosomes in the allergic murine model

Sajad Dehnavi[1], Mahvash Sadeghi[2], Ata A. Ghadiri[2], Afshin Amari[2], Mohammad Ghasemi Dehcheshmeh[2], Ali Asadirad[2]

1. Mashhad University of Medical Sciences, Mashhad, Iran
2. Ahvaz Jundishapur University of Medical Sciences, Ahvaza. Iran

Background: Allergic airway disorders are among the most important concerns worldwide. Allergen-specific sublingual immunotherapy (SLIT) was introduced as a noninvasive alternative route for established subcutaneous immunotherapy (SCIT). Mesenchymal stem cell (MSC)-derived exosomes are considered to be attractive delivery systems with immunomodulatory potential. Hence, the current study aimed to investigate the potential of ovalbumin (OVA)-enriched MSC-derived exosomes through a sublingual route as a prophylactic regimen in a murine model of OVA-induced allergic airway inflammation.

Methods: MSCs were harvested from murine abdominal adipose tissue via enzyme digestion. MSC-derived exosomes were isolated and OVA-enriched exosomes were prepared through the incubation method. Healthy Balb/c mice received sublingual OVA-enriched exosomes twice a week for three consecutive weeks. Next, mice were sensitized via intraperitoneal injections and nebulizer challenges. Then, mice were sacrificed, TGF-β secretions by splenocytes were analyzed via ELISA, and eosinophil infiltrations were assessed in nasal lavage fluid (NALF).

Results: Prophylactic regimen via OVA-enriched MSC-exosomes containing 10µg OVA effectively suppressed allergic sensitization through a significant increase in TGF-β levels and reduce in the eosinophil count in NALF compared to control groups which received PBS, free OVA, and free MSC-exosome prior to sensitization.

Conclusion: The results indicated that MSC-exosomes efficiently act both as a delivery system and as an immunomodulatory agent to suppress allergy induction and NALF eosinophil infiltration via inducing regulatory responses of Tregs.

Keywords: Allergen-specific sublingual immunotherapy (SLIT), Mesenchymal stem cell (MSC), exosome, ovalbumin (OVA)

Investigating the history of allergies in suspected and confirmed eosinophilic esophagitis patients and their families

Saba Ebrahimi[1], Saghi Khatami[1], Mehrnaz Mesdaghi[2]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Allergy and Clinical Immunology, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Eosinophilic esophagitis (EoE) is the most common eosinophilic gastrointestinal disorder (EGID), characterized by the infiltration of large numbers of eosinophils into the esophageal epithelium and associated clinical symptoms. Esophageal biopsy of patients with suspected EoE may not show a high number of eosinophils, therefore, a final diagnosis is not made for this group of patients. Since a history of allergies has been reported in many patients with confirmed EoE or their families, the presence of a history of allergy in suspected EoE patients may be helpful for diagnosing these patients. The aim of this study is to investigate the history of allergies in confirmed and suspected EoE patients.

Methods: A total of 30 patients with symptoms and endoscopic findings matching the EoE diagnosis criteria were included in this study. 15 confirmed EoE patients whose eosinophil count in their biopsies was equal to or greater than the diagnosis threshold (≥15 eosinophils per high power field (Eo/HPF)), and 15 suspected EoE patients with eosinophil count less than the threshold number (<15 Eo/HPF). The allergy records were collected by reviewing patients' medical reports and filling out questionnaires.

Results: 73.3% of confirmed EoE cases and 41.7% of suspected EoE cases had a history of food allergy. 60% of confirmed and 33.3% of suspected EoE cases had a history of other allergic diseases. Furthermore, 73.3% and 58.3% of confirmed and suspected EoE patients had a history of allergic disease in their family members, respectively. There was no significant difference (p>0.05) between the two groups regarding allergy history.

Conclusion: In both confirmed and suspected EoE gropes a considerable number of cases had a history of allergies and there was no significant difference between the two groups in this regard. This fact can indicate the similarity between these two groups and may lead to a definite diagnosis of this disease in the case of suspected EoE patients.

Keywords: Food allergy, Allergic diseases, Eosinophils, Diagnosis

Investigation of Tea grass extract on the expression of GATA3 ،T-bet ،Foxp3 genes in balb/c mice with allergic asthma

Fahimeh Rostamabadi[1], Reza Nosratabadi[2], Amir Rahnama[3], Vahid Mohammadi Shahrokhi[4]

1. Department of Medical Immunology, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
2. Department of Medical Immunology, Afzalipour Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
3. Department of Non-Communicable Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
4. Immunology of Infectious Diseases Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

Background: Asthma is a chronic inflammatory disease of the respiratory tract that is associated with overreaction and reversible narrowing of the airways. This inflammation causes clinical symptoms such as wheezing, shortness of breath, and cough. Herbal medicine and its therapeutic effects have already been known. Tea grass has been used in the treatment of many diseases due to its anti-inflammatory effects. This study was performed to investigate the effects of Tea grass on the inflammatory responses in mice with allergic asthma.

Methods: This study was performed on BALB/c mice. Thirty mice were divided into 5 groups: 1. non-asthmatic control group (control), 2. Untreated asthmatic group (asthma), and 3-5. Asthmatic animals treated with, Tea grass at different doses. For induction of asthma, BALB/c mice were sensitized with subcutaneous injection and inhalation of ovalbumin. The effects of Tea grass on mRNA expression of T-bet, GATA3, and FOXP3 were determined by quantitative real-time PCR (qPCR).

Rsults: The results of the present study showed that there was a significant increase in T-bet and FOXP3 expression in the asthma group compared to the control group (p<0.001). There was also a significant decrease in the level of GATA3 in the group treated with 150 mg/kg HP compared to the asthmatic group (p<0.01).

Conclusion: The extract of Tea grass in a specific dose reduced GATA3 and increased T-bet and FOXP3 expression in asthmatic mice. Which, improved the symptoms of the disease.

Keywords: Asthma, Tea grass, GATA3, T-bet, Foxp3

Modulating Th1/Th2 balance by aptamer-modified ovalbumin-enriched mesenchymal stem cell-derived exosomes in the allergic murine model through sublingual immunotherapy

Mahvash Sadeghi[1], Sajad Dehnavi[1], Ata A. Ghadiri[1], Ali Khodadadi[1], Moosa Sharifat[1], Ali Asadirad[1]

1. Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Mashhad University of Medical Sciences, Mashhad, Iran

Background: The prevalence of allergic asthma has increased during recent decades. Noninvasive procedures such as sublingual immunotherapy (SLIT) attracted much attention and are introduced as an alternative to subcutaneous immunotherapy (SCIT). Mesenchymal stem cell (MSC)-derived exosomes are introduced as interesting delivery systems with immunomodulatory potential. In addition, administrating molecular targeting agents such as aptamers (Apt) have been widely applied for targeted drug delivery. Therefore, this study aimed to evaluate the effects of aptamer-modified ovalbumin-enriched mesenchymal stem cell-derived exosomes to improve SLIT.

Methods: MSC isolation was conducted through enzyme digestion from murine abdominal adipose tissue. MSC-derived exosomes were isolated. Apt-modified exosomes were prepared via EDC/NHS chemistry. Then, OVA enrichment was conducted through the incubation method. Following the sensitization process in Balb/c mice, SLIT was performed by OVA-loaded Apt-exosomes twice a week for two months. Next, IFN-γ and IL-4 production by spleen cells was assessed by ELISA, and lung tissues underwent histopathological analysis.

Results: SLIT using OVA-loaded Apt-exosomes significantly reduced IL-4 secretions while increasing IFN-γ production compared to control groups. In addition, local inflammation and limited peri-bronchiolar and perivascular infiltrations were observed in the lung tissue of mice who received Apt-OVA-MSC exosomes.

Conclusion: The results indicated anti-inflammatory effects of Apt-OVA-MSC exosomes as a result of successful Th2-to-Th1 shift in immune responses.

Keywords: Sublingual immunotherapy (SLIT), Mesenchymal stem cell (MSC), exosome, aptamer

Multistrain Probiotics Supplement Ameliorates Asthma Symptoms via Increasing Treg Cells Population and Modulation of Immune Responses: A Randomized, Double-Blind, Placebo-Controlled Trial

Tannaz Abbasi-Dokht[1], Sina Sadrifar[2], Rasoul Baharlou[1], Sarvenaz Forouzandeh[1], Maral Hemmati[1]

1. Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
2. Department of Internal Medicine, Kosar Hospital, Semnan University of Medical Sciences, Semnan, Iran

Background: The favorable effects of probiotics have been demonstrated in allergic disorders. However, the underlying immunological mechanisms are poorly understood. In the present study, we investigated the improvement of clinical symptoms and immunological balance after receiving probiotics in patients with asthma.

Methods: The present study was a randomized, double-blind, placebo-controlled trial that 40 patients with asthma were enrolled. They were treated with probiotics or a placebo: 1 capsule/day for 8 weeks. Pulmonary function test, percentage of CD4+ CD25+ FoxP3+ Tregs, and gene expression of T-bet, GATA-3, RORγt, and Foxp3 in PBMCs were assessed at baseline, and after treatment.

Results: Our results showed a significant increase in the expression of FoxP3 and CD4+ CD25+ FoxP3+ Tregs population, while, RORγt and GATA3 expression were reduced. In addition, pulmonary function tests showed a significant improvement in Forced Expiratory Volume and Forced Vital Capacity after receiving probiotics.

Discussion/Conclusion: Our findings demonstrate that eight-week treatment with probiotic supplementation can control Th2-predominant and Th17 pro-inflammatory responses and improve Forced Vital and Forced Expiratory Volume in asthmatic patients. It seems probiotics can be used besides common treatments for patients with asthma.

Keywords: Asthma, Probiotic, Treg, T-bet, GATA-3, RORγt, Foxp3

Protecting the kidneys through reducing oxidative stress by long-term administration of sodium hydrosulfide in ovalbumin-induced chronic asthma

Farzaneh Kianian[1], Behjat Seifi[1], Mehri Kadkhodaee[1], Hamid Reza Sadeghipour[1], Mina Ranjbaran[1]

1. *Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

Background: Asthma is one of the most common chronic respiratory diseases worldwide. However, it has been reported that asthma inflammation is not limited to the lungs and may have devastating effects on distant organs. This study was designed to evaluate the beneficial effects of long-term administration of hydrogen sulfide (H2S) donor sodium hydrosulfide (NaHS) on markers of lung oxidative stress and airway remodeling to protect the kidneys during chronic asthma.

Methods: Male BALB/c mice were randomly divided into 3 groups (n = 5-7): control, asthma, and NaHS. Except for the control group, sensitization, and challenge were performed with ovalbumin. The NaHS group received 14 μmol/kg NaHS intraperitoneally 30 min before each challenge. 24 h after the last challenge, samples of bronchoalveolar lavage fluid (BALF) and lung and kidney tissues were collected.

Results: Induction of asthma significantly increased the levels of lung malondialdehyde (MDA) and BALF interleukin-13 (IL-13) and the scores of goblet cell hyperplasia and sub-epithelial fibrosis, and decreased lung superoxide dismutase (SOD) activity. In addition, asthma resulted in a significant increase in MDA levels and a significant decrease in SOD activity in the kidney tissues. NaHS administration significantly returned all of these indicators to the levels measured before sensitization and challenge.

Conclusion: In addition to improving asthma-induced lung injury through reducing oxidative stress and airway remodeling, NaHS administration improves asthma-induced kidney damage by reducing oxidative stress. Thus, H2S may have an important role in renal protection during asthma.

Keywords: Asthma, Sodium hydrosulfide, Kidney, Oxidative stress

Sublingual immunotherapy by mannose-decorated ovalbumin (Ova) encapsulated poly lactic-co-glycolic acid (PLGA) nanoparticles modulates immune responses in mouse models of allergy

Davide haghnavaz[1], safoora pordel[1], MohammadAli Rezaee[1], Saeideh Sadat Shobeiri[1], Mohammadreza Dashti[1], bahareh ansari[1], Mojtaba Sankian[1]

1. Immunology Research Center, Medical School, Mashhad University of Medical Sciences

Background: Allergen-specific sublingual immunotherapy (SLIT), is a non-invasive and effective treatment for type I respiratory allergies. In line with various strategies to increase the efficacy of SLIT, we assess ovalbumin (Ova) encapsulated poly lactic-co-glycolic acid (PLGA) nanoparticles targeted by mannose in this study.

Methods: The nanoparticles containing Ova were synthesized by emulsion/solvent evaporation method and attached to D- mannose. BALB/c mice have been sensitized to Ova and then treated in five paths: subcutaneously with free Ova (SCIT), sublingually with free Ova, Ova-PLGA NPs (two doses), Man-Ova-PLGA NPs (two doses) and Apt-Ova-PLGA NPs (high dose). Ova-specific IgE, IgG1, and IgG2a antibodies, IL-4, and IFN-γ levels were measured by ELISA for evaluation of immunologic responses. In addition, T cell proliferation was assessed by MTT.

Results: Our data displayed a significant decrease in Ova-specific IgE antibody, IL-4, and T cell proliferation in NPs treated mice which the low dose of Man-Ova-PLGA NPs showing the most decrease. Also, IgG2a antibody and IFN-γ levels had the most rise in the low-dose Man-Ova-PLGA NPs treated mice. However, the IgG1 antibody level did not show any alteration among groups.

Conclusion: The results revealed that Man-Ova-encapsulated PLGA NPs could achieve a reduction of the required allergen doses, thus it proposes an effective functionalized delivery system for SLIT improvement.

Keywords: SLIT, PLGA NPs, mannose, respiratory allergic diseases

Sublingual immunotherapy by nanofibers containing allergen and curcumin in mic model of allergy

Bahareh Ansari[1], Navideh Haghnavaz[1], Saeideh Sadat Shobeiri[1], Safoura Pordel[1], MohammadAli Rezaee[1], Ayda Estaji[2], Mojtaba Sankian[1], Maryam Hashemi[3]

1. Immunology Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
3. Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Allergen-specific sublingual immunotherapy is recognized to be an efficacious and well-tolerated treatment for preventing and treating aspiratory airway allergic diseases. Nanofiber-based dosage forms have been considered potential drug delivery systems due to controllable pore structures and high surface-to-volume ratio, making them ideal for improving the extent and rate of drug dissolution. The aim of this study was to investigate the combined therapeutic effect of curcumin and ovalbumin in free form and nanofiber-based in order to increase the effectiveness of sublingual immunotherapy with ovalbumin in a mouse model of rhinitis allergy.

Methods: Nanofibers containing curcumin (CUR), ovalbumin (OVA), or both were successfully prepared via electrospinning and characterized. To establish ovalbumin (OVA)-induced rhinitis allergic models, BALB/c mice were sensitized with ovalbumin. Afterward, SLIT with free curcumin (2.5 and 5 mg), Ovalbumin (5 mg), or nanofibers containing curcumin and Ovalbumin were carried out and immunological profiles were evaluated. For assessment of immunologic responses, T cell cytokine, IL-4, and Serum levels of immunoglobulin E (IgE) were measured by ELISA, and T cell proliferation was evaluated by MTT. In addition, lung and nasal histological examinations and nasal lavage fluid (NALF) cell counting were carried out.

Results: SLIT treatment with all nanofibers lead to significantly decreased specific IgE. The low-dosage combination immunotherapy by curcumin and ovalbumin nanofiber forms (N.CUR 2.5-OVA), showed a decreased level of IL-4 compared to other treated groups. The assessment of NALF showed a significant decrease in specific cell and eosinophil counts in the treated nanofibers groups. The histopathological results of NAL in the optimal formulations were normal with no cellular infiltration and no inflammation.

Conclusions: It was shown that SLIT with curcumin and ovalbumin-nanofibers-combination therapy can improve and suppress the Th2 immunomodulatory responses, therefore, can be considered as potential immune modulatory agent.

Keywords: immunotherapy, SLIT, allergy, nanofibers

Treadmill exercise restores memory and hippocampal synaptic plasticity impairments in asthmatic juvenile rats

Narges Marefati[1], Amin Mokhtari-Zaer[2], Hassan Ghoshooni[3], Mohammad Hossein Boskabady[4], Mahmoud Hosseini[4]

1. Dept. of Physiology and Medical Physics, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Torbat Jam Faculty of Medical Sciences, Torbat Jam, Iran
3. Dept. of Physiology and Medical Physics, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
4. Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Asthma is a common chronic inflammatory respiratory disease in childhood. Previous studies demonstrated that the prevalence of asthma has plateaued or may be declining, however, it is still a major public health problem among young children. The pathophysiological features of allergic asthma are characterized by an imbalance in the T helper type 1/T helper type 2 (Th1/Th2) cytokines ratio, with enhanced Th2 and suppressed Th1 immune responses. Th2 response is linked with interleukin (IL)-4, IL-5, and IL-13 release to contribute to inflammatory cell influx, airway remodeling, and airway hyper-responsiveness, while the Th1 response is associated with the production of interferon- γ (IFN-γ) and IL-2. Studies demonstrate that asthma, especially during childhood, affects the brain's functions, including learning and memory. Exercise is well known for its neuroprotective functions and for its beneficial effects on asthma. We aimed to assess the effects of exercise on cognitive function, synaptic plasticity, and hippocampal brain-derived neurotrophic factor (BDNF) levels in ovalbumin (OVA) sensitized juvenile rats.

Methods: Rats were sensitized (asthmatic) by intraperitoneal administration and inhaled OVA. Animals were subjected to treadmill running exercise during the OVA-challenged period. T-helper type 2 (Th2) cytokine [interleukin (IL)-4], Th1 cytokine (INF-γ) levels, and INF-γ/IL-4 (Th1/Th2) ratio in bronchoalveolar lavage fluid (BALF), and tracheal response to methacholine and OVA were measured. Further, memory behaviors and BDNF levels were measured in the hippocampus as well as long-term potentiation (LTP) was assessed by recording field excitatory postsynaptic potentials (fEPSPs) in the hippocampus.

Results: The levels of IL-4 and TGF-β were decreased but INF-γ level and INF-γ/IL-4 ratio increased in the BALF due to exercise in the OVA-sensitized animals. In addition, exercise improved OVA-sensitization-induced cognitive impairments, increased BDNF levels, and enhanced hippocampal LTP in OVA-sensitized rats. Exercise is not only effective in alleviating airway inflammation by restoring Th1/Th2 cytokines balance but also is a candidate for the improvement of memory and synaptic plasticity deficits partially through increasing the levels of hippocampal BDNF in OVA-sensitized rats.

Keywords: Exercise, Asthma, Ovalbumin, BDNF

Vitamin D regulates GATA3 gene expression in allergic asthma

Saiedeh Erfanian[1], Maliheh Moradzadeh[2], Abdolreza Sotoodeh Jahromi[3], Simin Jafaripour[4]

1. Department of Advanced Medical Sciences and Technologies, Research Center for Non-Communicable Diseases, Jahrom University of Medical Sciences, Jahrom, Iran
2. Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran.
3. Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, Iran
4. Department of Biology, University of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada

Background: Asthma is becoming a major health problem in many countries. Immune responses in allergic asthma, as the most prevalent asthmatic phenotype, are mediated mostly by a subtype of T lymphocytes referred to as the effector lineage of Type 2 Th cells (Th2). The development of Th2 cells is mainly governed by a zinc finger transcription factor, i.e., GATA-binding protein3 (GATA3). Allergic asthma is a complex disease, and vitamin D deficiency has been named as a non-genetic risk factor for its development. Vitamin D, a steroid hormone belonging to the family of nuclear receptors, has shown significant immunosuppressive effects in previous studies.

Methods: In this study, given its immunomodulatory properties, we aimed to investigate the effects of different concentrations of vitamin D on GATA3 gene expression in peripheral blood mononuclear cells (PBMCs), including Th2 cells, and compare GATA3 expression levels between PBMCs taken from allergic asthmatic patients and healthy controls.

Results: The total sample size was 40 and the quantitative real-time PCR (qPCR) procedure was applied to assess the mRNA expression levels of GATA3 in different groups. Collectively, our results demonstrated that the expression of GATA3 in PBMCs taken from patients with allergic asthma is lower than that from healthy controls. In addition, in the control group, cells co-cultured with vitamin D had a significantly increased GATA3 expression. However, in the patient group, such an increase was only observed in cells treated with 10-7M-vitamin D. By contrast, incubation with vitamin D at the concentration of 10-6 M slightly decreased the expression of GATA3 among patients.

Conclusion: In summary, it is likely that vitamin D should regulate GATA3 gene expression in the PBMCs in a dose-dependent manner. The impacts of this steroid hormone can also differ between the status of health and allergic asthma in either extent or direction.

Keywords: allergic asthma, immune response, GATA3, Th2, vitamin D

Prevalence of respiratory allergy among university students of Jundishapur University of Medical Sciences, Ahvaz

Sahar Molavi Nia[1]

1. Department of Medical Mycology, Faculty of Medical Sciences, Jundishapur University of Medical Sciences, Ahwaz, Iran.

Background: The prevalence of allergic diseases in the world and also in Iran is increasing. Allergens play an important role in causing these diseases. Since respiratory allergens are the most important part of allergens, this study was conducted to determine the prevalence of respiratory allergies among students of Jundishapur University of Medical Sciences in 2019.

Methods: This descriptive-analytic study was conducted on those students who were studying at the moment in Jundishapur University of medical sciences in 2019. According to Morgan table 515 of the sample size was specified. Based on the ratio of students in each department, data was gathered. For those who participated in our study, the questionnaire was completed. Information such as sociodemographic characteristics (age, gender, nationality, major of study, exe), respiratory allergy, and family history of allergies was collected. Descriptive analysis was performed by using SPSS version 22. The Chi-square test was used to test for any association between allergies and mentioned variables.

Results: The result showed that there was an association between respiratory allergy and gender and family history. However, no association was seen between the month of birth and the field of study.

Conclusion: Respiratory allergy is one of the problems that occur for various reasons, and also it has Side effects, annoying symptoms of this allergy, Heavy medical costs, and decreasing quality of life. As a result, similar studies should be conducted in this region because of its special geographical properties and also reduce social, mental, and economic effects caused by disease.

Keywords: Allergy, respiratory allergy, student

Airborne Asteraceae pollen monitoring and relationship whit meteorological data: The main cause of the potential photodermatitis and skin allergy in Tehran, Iran

Maryam Sharif Shoushtari[1], Haleh Abdoli Sereshki[1], Ahmad Majd[2], Mohammad Nabavi[3], Mostafa Moin[4], Mina Mohammadrezaei[1], Mina Noroozbeygi[1], Mohammad-Ali Assarehzadegan*[1]

1. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran 2. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Plant Biology, Faculty of Biology Science, Islamic Azad University, North Tehran Branch, Tehran, Iran
3. Department of Allergy and Clinical Immunology, Rasool-E-Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
4. Department of Immunology and Allergy, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Plant-associated dermatitis can be categorized into Phyto dermatitis, irritant contact dermatitis, contact urticaria, and allergic contact dermatitis (ACD). The Asteraceae family is one of the largest in the world, with members across all continents. The seasonal distribution and airborne predominance of Asteraceae pollen in Tehran are poorly known. In this research, we investigated the longitudinal distribution of Asteraceae pollen using an aerobiological dataset. Additionally, the impact of meteorological conditions on pollen from Asteraceae species was investigated.

Methods: Asteraceae pollen was collected using the static Durham method during a 4 yrs (Jan 2019-Jan 2023) aerometric surveillance program in Tehran, Iran. Microscope counts were converted into atmospheric concentrations and expressed as pollen grains/cm^2. Meteorological parameters were obtained from the State Meteorological Service. Skin prick test (SPT) in patients attending allergy clinics Rasoul-Akram Hospital using commercial extracts conducted.

Results: Twenty-one pollen types representing the native, as well as the introduced Asteraceae plants, with a relatively low daily mean concentration, were observed for 4 years. The highest pollen concentrations were reached by Artemisia spp. (Mugwort), followed by Taraxacumspp. (Dandelion), Iva ciliata (Annual marsh elder), Chrysanthemum maximum (Shasta daisy), Xanthium spp. (Common cocklebur), Helianthus spp. (Sunflower), Achillea spp. (Yarrow), Bellisperemis (English daisy), Gazania spp. (Treasure flower), Tragopogon spp. (Goats beard), Echinops spp. (Globe Thistles),Zinnia spp.(Common zinnia), Dahlia spp. (garden dahlia), Rudbeckia spp. (Black-eyed-susans), Tagetes spp. (Marigolds), Coreopsis spp. (Calliopsis), Acroptilon Cass (Russian knapweed), Solidago virgaurea (European goldenrod), Parthenium hysterophorus (whitetop weed), Cineraria maritima (Silver ragwort), and Chrysanthemm spp. (Daisy). The most important genera in the air are Artemisia, between daily Asteraceae pollen concentrations and daily mean temperature observed during each sampling year. SPT revealed a comparatively higher degree of sensitization among 200 patients, who were sensitized to mugwort pollen 82 (41.5%), respectively.

Conclusion: Asteraceae are essential for comprehending immunological responses for phytodermatitis and a promising technique to enhance allergy diagnosis and treatment is the Asteraceae allergy screening panel. Future clinical investigations should describe the role of Asteraceae pollen, in allergy sensitization and respiratory morbidity given the public health burden associated with personal Asteraceae pollen exposure in other geographical areas.

Keywords: Allergy, Aerobiology, Asteraceae, Phytodermatitis

Association of allergic diseases and seizures in children

Homa Sadri[1], Negar Toami[2], Marzieh Tavakol[3], Hadi Montazer Lotf Elahi[4], Ehsan Zahmatkesh[4]

1. Non-Communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran
2. Department of Pediatrics, Imam Ali Hospital, Alborz University of Medical Sciences, Karaj, Iran
3. Non-Communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran
4. Department of Pediatrics, Imam Ali Hospital, Alborz University of Medical Sciences, Karaj, Iran

Background: Allergy and epilepsy are syndromes of heterogeneous diseases with likely multifactorial origins. It is hypothesized that inflammation from allergic diseases may predispose children to seizures but little is known about the relationship between allergy and seizures. The aim of this study is to investigate the frequency of seizure disorders in children with asthma and allergy.

Methods: Cross-sectional survey study of parents of 1300 children and adolescents under 20 years of age referred to the Allergy and Asthma Clinic of Imam Ali (AS) Karaj Hospital who were asked to complete a screening questionnaire for seizures in their children. Parents who reported any history of seizures in their children were contacted to answer a second in-depth questionnaire to determine more detail about the type, triggers and treatment of seizures.

Results: A total of 705 males (62%) and 433femalese (38%) participated in this study (median age: 6.62±4.57 years) and 70.6% of them had asthma, 15.2% allergic rhinitis, 5.6% atopic dermatitis,3.5% urticaria,2.7% food allergy,1% drug allergy and 1.4% other allergic disease.88 patients(7.7%) had a history of a doctor diagnosis of seizure. 57 patients(5%) had febrile convulsion and 15 ones(1.31%) had idiopathic epilepsy. None of the febrile convulsions, ever seizures, and epilepsy were significantly related to the type of allergic diseases but there was a significant association between the number of allergic diseases in patients with idiopathic epilepsy (p=0.007) and febrile convulsion (p=0.000). Also, there was a significant association (p=0.037) between febrile convulsion and a history of allergic disease in the patient's mothers. The relation between the number of allergic diseases in parents and idiopathic epilepsy in their children wasn't significant (for mothers p=0.052 and for fathers p=0.848)

Conclusions: Atopic background in children is related to both idiopathic epilepsy and febrile convulsion, and in the meantime, the mother's genetic background may play a more effective role than the father's.

Keywords: Allergy, seizure

Bee venom allergy and risk factors for systemic allergic reactions among beekeepers in the south of Kerman province

Fatemeh Raeesi Nejad[1], Nasrin Bazargan Harandi[2], Milad Shahriari[3], Alireza Raeesi Nejad[4]

1. Department of Immunology, School of Medicine, University of Jiroft Medical Sciences, Jiroft, Iran
2. Department of Allergy and Immunology, School of Medicine, University of Kerman Medical Sciences, Kerman, Iran
3. Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, University of Jiroft Medical Sciences, Jiroft, Iran
4. Department of Microbiology, Islamic Azad University of Sirjan, Sirjan, Iran

Background: The severity of reactions to bee stings varies from mild local reactions to systemic allergic reactions (SARs) including anaphylaxis, which can lead to death. Beekeepers are at especially high risk for developing an allergic reaction to bee venom due to their persistent exposure to bee stinging. This study aimed to evaluate the characteristics of sting reactions and the risk factors for developing SARs among beekeepers in the south of Kerman province.

Methods: With the collaboration of the Beekeepers Association, a self-reported questionnaire was applied to 530 beekeepers in seven different cities in the south of Kerman province. Descriptive methods were used to analyze demographic data, and risk factors for SARs were determined by a logistic regression model.

Results: There were 231 responses to the questionnaire of which 83% were from male beekeepers; the median age was 45.5 years. Beekeepers reported 5.81 % SARs to bee stings, 70.22 % reported mild local reactions, 4.19% large local reactions and 19.78 % had no reactions to bee stings. The main factors that predisposed beekeepers to SARs were a family history of asthma, having a family member with bee venom allergy, seasonal rhinitis, and beekeeping duration. However 92.32 % of the beekeepers were aware of the possible lethal effects of bee venom, and only 16 % recognized the management of an anaphylaxis reaction.

Conclusion: Bee venom allergic reactions can be fatal and beekeepers are at high risk of systemic reactions. Therefore awareness of the prevalence of these reactions and implementation of educational and training programs about anaphylaxis symptoms, immunotherapy, and practical instructions on when and how to use an epinephrine auto-injector is essential for beekeepers and healthcare professionals.

Keywords: Beekeepers, Bee Sting, Venom Allergy, Systemic Allergic Reactions

Chronic urticaria: seeking the cause in Iranian population

Sepideh Darougar[1]

1. Department of Pediatrics, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

Background: Chronic urticaria is the recurrence of erythematous itching papular skin lesions for at least 6 weeks. Although it is often considered a benign condition, there are reports of the association of chronic idiopathic urticaria with infections, thyroid disease, foods, medications, autoimmunity, neoplasms, and low serum level of vitamin D. We aimed to identify the effect of lifestyle characteristics, environmental factors, family history, and specific comorbidities as potential external eliciting factors on the onset of chronic urticaria.

Methods: The present research was conducted on 141 adult patients diagnosed with chronic urticaria at the allergy clinics of Azad University hospitals in Tehran from Jan. 2021 to Jan. 2022. A questionnaire on different life events during the past 3 months prior to the onset of urticaria was developed to evaluate the characteristics of the patients with chronic urticaria. Meanwhile, 58 healthy individuals who were similar to the patients in terms of age range and gender were asked to participate in the study as the control group.

Results: The mean age of the patients and the controls were 41.7 years and 39.8 years, respectively. The female and male patients were 105 (74.47%) and 36 (25.53%), respectively. Among the evaluated predisposing factors, only two were meaningful, including the use of new medications (23.4% versus 19%; p= 0.006) and the co-existence of anxiety and depression (14.1% versus 4%; p=0.036).

Conclusion: Among the different factors evaluated in this study, only two were found to be significant in relation to chronic urticaria: anxiety/depression and medications.

Keywords: Chronic urticaria, spontaneous urticaria, idiopathic urticaria

Effects of Zataria Multiflora Extract on Cytokines Expression in Allergic Rhinitis Patients: a Double-Blind Randomized Clinical Trial

Farahzad Jabbari-Azad[1], Nazila Ariaee[1], Yaser Yadegari[1], Mohamad Shabestari[2], Javad Asili[3], Maryam Panahi[4], Jalal Ghorbani[1]

1. Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Preventive Cardiovascular Care Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Department of Emergency Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Allergic rhinitis is a common inflammatory disease of the nasal mucosa. Cytokines such as interleukin-4 (IL-4), interleukin-5 (IL-5), and Interferon-gamma (IFN-y) play a critical role in this inflammatory process. Zataria multiflora (ZM) is an herbal product with proven antioxidant and anti-inflammatory effects. Accordingly, in this study, the effect of ZM extract on allergic rhinitis patients was evaluated by measuring IL-4, IL-5, and IFN-y cytokines before and after the treatment.

Methods: In this double-blind randomized clinical trial, thirty allergic rhinitis patients were randomly allocated into two groups to receive either the thyme placebo (group A) or the product (group B). The samples consisted of 10 ccs of intravenous blood taken from patients before and two months after the intervention. The concentration of IL-4, IL-5, and IFN-γ was assessed in the serum samples and culture supernatants by a commercial ELISA kit. Patient symptoms were also monitored throughout the study using the Sino Nasal Outcome Test (SNOT-22). Data was analyzed in IBM-SPSS v.22.

Results: It was observed that the ZM alleviates nose-related symptoms and improves sleep state more than the placebo. After the treatment, the IL-4 level was significantly higher in the case group compared with the control group (*p*-value: 0.046). Moreover, a significant difference in IL-5 was reported between the two groups before the treatment. After the intervention, IL-5 levels decreased in both case and control groups; however, there were no significant differences between the two groups regarding IL-5 levels. There was no statistical difference in IFN-γ level during the study.

Conclusion: This study illustrated the alleviating effects of Zataria Multiflora on allergic rhinitis patients, probably via increasing IL-4 and decreasing IL-5 expressions. Thus, it is suggested to consider thyme in addition to the conventional treatment options for allergic rhinitis.

Keywords: Allergic rhinitis, Cytokines, IL-4, Shirazi thyme, Zataria multiflora

Evaluation of disease-modifying therapies influence on IgE titer and allergy condition in Multiple Sclerosis patients

Sina Nouri[1], Sadegh Feizollahzadeh[1]

1. Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Urmia University of Medical Sciences, Urmia, Iran

Background: Developing Multiple Sclerosis (MS) and allergy in one patient while they possess contradictory immune-dominant responses is open to question. Furthermore, the prescription of disease-modifying therapies and immune-suppressive medications influences complex immune-process associated with allergy and IgE production directly or indirectly. The purpose of the present research was to evaluate the effect of disease-modifying therapies and immune-suppressive therapies on total IgE titer and allergy conditions in MS patients.

Methods: The research was designed as a descriptive and cross-sectional study. 124 people who are diagnosed with MS participated in this study. History of allergy, allergy-related symptoms, and medication used in addition to demographic information was elicited by interview then, the venous blood sample was collected for the subsequent step. Sandwich Enzyme-linked immunosorbent assay (ELISA) was the adopted method to analyze total IgE.

Results: The prevalence of allergy among patients, based on questionnaire data, was 44.3% (55 individuals) nevertheless, laboratory findings showed, it was 14.5% (18 individuals, have a total IgE titer of more than 50 IU/ml). The number of patients who used drugs for MS treatment was 113 (91.2%) and 11 (8.8%) did not use any chemical drugs. The mean total IgE titer of patients who took MS medication and those who stopped taking the medication did not differ significantly and were 31.04 ± 27.04 and 31.02 ± 28, respectively. The average IgE titer was different in the groups of patients who used different drugs, but it was not statistically significant. Patients using interferon beta, glatiramer acetate, fingolimod, rituximab, teriflunomide, and dimethyl fumarate had average serum levels of 29.66, 54.91, 21.15, 21.08, 19.93, and 19.93.

Conclusion: Although there was not a statistically significant between the two groups of patients who used- and non-used medications, observation of total IgE difference among used-medication groups suggests the idea that probably, disease-modifying therapies influence the titer of IgE and allergy condition, subsequently.

Keywords: Multiple Sclerosis, Allergy, disease-modifying therapy, IgE, and Multiple Sclerosis

Evaluation of the difference between children before school age with asthma in rural and urban areas in the West Azerbaijan province of Iran

Hamidreza Houshmand[1]

1. Assistant Professor of Allergy and Clinical Immunology, Department of Pediatrics, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran

Background: Allergic disorders are one of the most critical health problems in the world. Due to the costs imposed on health systems, much effort is being made to prevent and control it. The prevalence of allergic disorders varies according to different countries and regions. Asthma is a chronic recurrent disease in children. The disease is estimated to grow to 400 million worldwide in 2025. The prevalence of asthma in Iran is reported to be 5.5%. Asthma prevalence is generally lower in rural than urban areas. However, specific rural exposures are thought to protect against the development of asthma among children with asthma. We examined childhood asthma prevalence and related conditions along an urban-rural gradient.

Methods: A total of 149 children, 85 boys (40.3%) and 61 girls (59.7%) with asthma, were identified in this study. Through a checklist in the form of a questionnaire (consisting of information about demographics, parental asthma, exposure to farm animals, pets, and tobacco, adequate home air conditioning, use of antibiotics during the first year of life, maternal gestational age, type of delivery, birth weight, age of onset of wheezing, length of breastfeeding, number of siblings,) which was filled by the parents of the children and after that, it was used as a data collection tool. The statistical significance level was considered less than 0.05. Data were analyzed by using SPSS 21 software.

Results: Out of these 149 children diagnosed with asthma, 25.3%, equal to 37, have parents with asthma. Furthermore, people had healthy parents, equal to 109 (72.7%). The most relative environmental factors affecting pediatric asthma were antibiotics (44.6%), animal exposure with 28.4%, and smoking 27% in urban areas. In rural areas, the most efficacious environmental factor on pediatric asthma was antibiotics with 62.5%, followed by exposure to animals with 48.6% in the second place, and finally tobacco smoke with 27.8% in the last place.

Keywords: Asthma, Risk Factors, Rural Areas, Urban Areas, Children

Evaluation of the Neutrophil to Lymphocyte Ratio in the Patients of Kawasaki Disease Resistant to Intravenous Immunoglobulin (IVIg) Therapy

Hamidreza Houshmand[1], Javad RasouIi[2]

1. Assistant Professor of Allergy and Clinical Immunology, Department of Pediatrics, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
2.Assistant Professor of Epidemiology, Department of Biostatistics and Epidemiology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran (Corresponding Author)

Background: Kawasaki disease is a systemic vasculitis of unknown etiology that is common in pediatric patients. The primary therapeutic strategy includes aspirin and Intravenous Immunoglobulin. The present study aimed to investigate the Neutrophil Lymphocyte Ratio (NLR) in patients with Kawasaki and its relationship with resistance to IVIg therapy.

Methods: The present study included the patients presenting to the Motahari Hospital, Urmia, Iran, who was diagnosed with Kawasaki disease and received IVIg during 2008-2019. The authors collected the data from the patient's medical records and reassessed the patients' data for meeting the diagnostic criteria for Kawasaki. Afterward, the data of eligible patients were entered into the analysis.

Results: The data from 460 patients were obtained and assessed for meeting the diagnostic criteria for Kawasaki. Of 460 patients, 241 met the eligibility criteria, and the data for other patients that meet the exclusion criteria were excluded from the study. According to the results of our study, response to IVIg therapy had a significant relationship with the variables of blood and urinary leukocyte counts (p=0.013 and p=0.01, respectively). However, we didn't find any significant relationship between the response to IVIg therapy and the variables of age, gender, NLR, neutrophil count, lymphocyte count, C - reactive protein (CRP), serum albumin level, ALT, hemoglobin, platelet count, and the interval between onset of symptoms and treatment initiation.

Conclusion: High blood leukocyte count along with low urinary leukocyte count can predict the response to IVIg treatment and subsequent prognosis in the patients affected by Kawasaki disease. However, the NLR did not show clinical relevance. Keywords: Kawasaki Disease, Vasculitis, Neutrophil to Lymphocyte Ratio, IVIg therapy.

Keywords: Kawasaki disease, Vasculitis, Neutrophil to Lymphocyte Ratio, IVIG Therapy

Immunotherapy in allergic rhinitis: its effect of on the immune system and clinical symptoms

Samaneh Kouzegaran[1]

1. Faculty of medicine, Iran

Background: Allergic rhinitis is one of the most common allergic diseases and is characterized by sneezing, rhinorrhea, nasal congestion, and nasopharyngeal itching. Subcutaneous immunotherapy (SCIT) for specific allergens is an effective treatment and induces an inhibitory effect of T regulatory lymphocytes and decreases clinical symptoms in allergic rhinitis. In this study effect of subcutaneous immunotherapy with specific allergens on clinical symptoms and T regulatory and T Helper cells cytokines, in patients with allergic rhinitis are evaluated.

Methods. In this study, 30 patients with moderate to severe allergic rhinitis according to clinical criteria and positive skin prick test for aeroallergens were selected and treated by SCIT. Clinical symptoms and T cells cytokines IL4, IL17, IFN gamma, TGF beta, GITR, FOXP3, and IL10 (by RT-PCR) were evaluated before and one year after initiation of treatment.

Results. 30 patients with allergic rhinitis in the age range 15-45 years old were treated by SCIT and 23 (14 female, 9 male) patients continued the study and 7 patients did not continue treatment. After immunotherapy, clinical symptoms decreased significantly. The specific cytokines TGF (p = 0.013) beta and IL10 levels (p=0.05) increased and changes were statistically significant. IL17 level was also increased, but not statistically significant. (p=0.8) IFN gamma, IL4, GITR, and FOXP3, all decreased, but the changes were not statistically significant (p<0.05).

Conclusion: Subcutaneous Immunotherapy for specific allergens decreases clinical symptoms in patients with allergic rhinitis and induces tolerance in T lymphocytes, especially by increasing T regulatory cells cytokines, TGF beta, and IL10.

Keywords: Allergic rhinitis; Subcutaneous Immunotherapy; cytokine

Increased regulatory T cells in the peripheral blood of children with eosinophilic esophagitis

Mahnaz Abdolahy[1]

1. Shahid Beheshty Medical Science University, Tehran, Iran

Background: Eosinophilic esophagitis (EoE) is an allergic inflammatory disease of the gastrointestinal tract. Regulatory T cells (Tregs) have a confirmed role in allergic disorders. Considering the allergic basis of Eosinophilic esophagitis (EoE), this study was conducted to evaluate peripheral blood Tregs in children with EoE.

Methods: Children with EoE, gastroesophageal reflux disease (GERD), and healthy controls (HC) (10 subjects in each group) were recruited after diagnosis by a pediatric gastroenterologist and allergist. After obtaining informed written consent, peripheral blood was obtained. Peripheral blood mononuclear cells were isolated by Ficoll gradient centrifugation. Flow cytometry was used to enumerate peripheral blood Tregs (CD4+CD25+FOXP3+ gated lymphocytes were considered as Tregs).

Results: CD4+ gated lymphocytes significantly increased in EoE and GERD groups compared to the HC group (p= 0.018). Tregs were significantly increased in EoE in comparison to the HC group (p=0.016). There were no statistically significant differences in Tregs of EoE as compared to GERD subjects (p=0.085).

Conclusion: Peripheral blood Tregs increase in patients with EoE as compared to healthy controls, which may be indicative of a feedback mechanism to regulate inflammatory responses.

Keywords: Regulatory T cells, Eosinophilic esophagitis, Gastroesophageal reflux disease, Peripheral blood mononuclear cells.

Investigating the prevalence of allergy to latex gloves in nursing students of Islamic Azad University, Bandar Abbas Branch

Afshin Mohebbi Zarin Dare[1], Zeinab Sadat Moosavifard[2]

1. Bachelor of Nursing student, Young and Elite Researchers Club, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran
2. Faculty, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran

Background: Latex allergy is one of the most common contact allergies in the hospital work environment. Some nurses and hospital staff have allergic reactions to materials produced from latex, such as latex gloves. Today, latex allergy is considered a debilitating disease and 5-15% of healthcare workers suffer from it. Some background history increases the likelihood of these reactions. The purpose of this study was to investigate the complications caused by the use of latex gloves and underlying factors in nursing students of Islamic Azad University, Bandar Abbas branch.

Methods: In this cross-sectional descriptive study, 150 nursing students studying at the Islamic Azad University, Bandar Abbas branch who frequently and daily use latex gloves in the hospital, in terms of different aspects of latex sensitivity, as well as medical records and underlying factors related to The occurrence of allergies such as hand dermatitis, atopy, and food allergy were investigated. In this study, the Medical University of South Carolina questionnaire was used to collect information.

Results: 150 people participated in this study, with an average age of 21.2 years and an average length of education of 3.3 years. 60.3% of people had experienced some degree of allergies caused by latex. In terms of medical records, 32.8% of people had a history of atopy, 41.5% had a history of allergies to various foods, and 22.1% of people had a history of hand dermatitis. The difference in the prevalence of skin and respiratory reactions among people with and without significant underlying factors were obtained.

Conclusion: In this study, the prevalence of skin and respiratory complications was higher than in similar studies. The best way to control latex allergy is to avoid products that contain latex. Due to the connection between allergic reactions caused by latex and some medical records, the medical selection of people at the beginning of employment and periodic monitoring of their health is absolutely necessary due to the mentioned problems.

Keywords: Nursing students, latex gloves, allergy, underlying factors

Knowledge, Attitude, and Practice of Pediatric Drug Allergy among Medical Interns

Elham Pourbakhtyaran[1], Noosha Samieefar[2], Meisam Akhlaghdoust[3], Saeed Sadr[2], Shahrzad Fallah[2], Narges Eslami[2]

1. MD-MPH, Department of Pediatric Neurology, Pediatric Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Allergy and Clinical Immunology, Mofid Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Functional Neurosurgery Research Center, Shohada Tajrish Neurosurgical Comprehensive Center of Excellence, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Drug allergy is a leading cause of mortality in hospitals. Several studies have demonstrated that healthcare providers lack sufficient knowledge. The aim of the present study is to evaluate the knowledge, attitude, and practice of medical interns.

Methods: A questionnaire was designed to investigate three aspects of knowledge, attitude, and practice toward drug allergy. Validity and reliability were checked by experts. A cross-sectional online survey was conducted among 350 medical interns at Tehran Universities. Data were analyzed by SPSS-25 software, and the level of significance was set as 0.05.

Results: A total of 346 complete questionnaires were received online. In this study, 57.2% of the participants were female, and the average GPA was calculated as 17.25. The scores obtained in the three areas of knowledge, attitude, and practice were at the average level. Knowledge score was significantly higher in participants who attended allergy wards or clinics (p=0.002). Based on the linear regression model with enter approach, the attitude was significantly associated with practice (p<.001).

Conclusion: According to the present study, attendance at allergy wards or clinics was significantly associated with increased levels of knowledge. Furthermore, a superior attitude was significantly associated with enhanced and satisfactory practice in the field of drug allergies. It is recommended to review and revise the medical curriculum to improve drug allergies education.

Keywords: Pediatric, Allergy, Drug Allergy, Adverse Drug Reactions, Medical Interns

Laboratory findings in a group of patients suffering from urticaria

Alireza Fereidouni[1], Mohammad Fereidouni[2]

1. Student Research Committee, Mashhad, Iran
2. Cellular and Molecular Research Center, Mashhad, Iran

Background: Urticaria is a common health problem that affects many people during their life and has a great negative effect on patients' quality of life. The etiology of this phenomenon is not well understood and many underlying diseases including allergies, autoimmunity, and metabolic diseases can cause urticaria. Different laboratory tests are recommended for identifying the causative disease. The aim of this study was to evaluate laboratory abnormalities among a group of patients with confirmed urticaria.

Methods: Patients who visited the allergy clinic of Birjand because of specialist-confirmed urticaria were enrolled in this study. Results of patients' laboratory tests were recruited from laboratory registry software.

Results: In total 570 patients were enrolled in this study. The average laboratory values for CBC, ESR, blood eosinophil count, and serum total IgE were (7.69 ± 2.06 1000/ul, 56.5 mM/H, 2.82 ± 2.20%, respectively). 35.1% of patients had high blood IgE. The most common abnormalities were positive ANA and anti-H.pylori IgG, and anti-thyroid peroxidase (39.7%, 15.9%, and 8.8%, respectively). None of the patients were positive for rheumatoid factor (RF) of hepatitis B antigen.

Conclusion: the results of this study show that about a third of patients with urticaria had high IgE or positive ANA. H pylori infection and thyroid autoimmunity were also among the common findings. Further studies need to clarify other possible causes for urticaria.

Keywords: Urticaria, Laboratory findings

Prevalence of specific IgE to different food allergens in sera of patients suspected to food allergies

Yazdan Ebrahimpour[1], Mohammad Fereidouni[1], Alireza Fereidouni[3]

1. Department of Immunology, Faculty of Medicine, Birjand University of Science, Birjand, Iran
2. Student Research Committee, Birjand University of Science, Birjand, Iran

Background: Allergy is a common health problem worldwide and many people suffer from different types of allergies, including food sensitivity. Identifying common and important allergens in each region is the first step for preventing and managing allergic disorders. The prevalence of sensitivity to food allergens is varied among different communities because of differences in nutritional habits and genetic background. The aim of this study was to evaluate the most prevalent food allergens among a group of patients in the east of Iran.

Methods: Sera of patients who visited the allergy clinic of Birjand University because of possible food allergies were used in this study. The level of specific IgE against thirty-three different food allergens was evaluated by a commercial immunoblot kit Specific IgE level above 3.5 IU/ml was considered positive.

Results: In total 641 samples including 109 children less than 6 years (mean age=2.66 ± 1.64 years, M/F ratio=0.52) and 532 cases older than 6 years (mean age=34.41 ± 16.05, M/F ratio=0.33) were evaluated. Among children samples, Wheat Flour and Egg White (both 4.76%) had the highest rate of sensitization. In the older group Citrus Mix, Tomato, Carrot, Celery, and Maize Flour were the most common food allergens (7.33%, 6.2%, 5.82, 5.63%, and 5.07% respectively). The following chart shows the prevalence of positive specific IgE in studies samples.

Conclusion: The results of this study showed that most cases had low levels of specific IgE against food allergens and just a very small minority had significant.

Keywords: Allergy, Food allergens, Specific IgE

Production and evaluation of the allergenicity of Aspergillus fumigatus extract

Saboura Nikpour[1], Mohammad Fereidouni[1]

1. Department of Immunology, Faculty of Medicine, Birjand University of medical science, Birjand, Iran

Background: Allergic disorders are common around the world. Most allergic patients are sensitized to one or several allergens, including inhalants or food allergens. Fungi are an important source of allergens and can trigger allergic symptoms, particularly asthma, and rhinosinusitis. Aspergillus fumigatus is one of the most common and important species, and its extract is routinely used in skin prick testing. The aim of this study was to produce an allergenic extract from Aspergillus fumigatus and evaluate its allergenic potential.

Methods: In this study, based on their clinical history and skin prick test, patients and controls were chosen. The protein concentration of the extract was measured by the Bradford method, and SDS-PAGE was performed to examine the protein pattern. A dot-blot assay was performed with the patient sera to detect serum IgE reactivity to allergens in Aspergillus fumigatus extract.

Results: The total amount of protein in the extract was 356 ug/ml. Different protein fractions were identified by SDS-PAGE, which included the most important allergens of Aspergillus fumigatus. A dot blot study confirmed the reactivity of the extract with serum from sensitized patients, similar to the commercial extract.

Conclusion: In the current study, a total extract of Aspergillus fumigatus was successfully produced, and its allergenicity was confirmed by several methods.

Keywords: Allergy, Aspergillus, Dot blot

Report of intense allergic rhinitis to Oleaceae pollen family in Tehran, IRAN

Haleh Abdoli Sereshki[1], Reza Falak[1], Maryam Sharif Shoushtari[1], Mohammad Nabavi[2], Mostafa Moin[3], Ahmad Majd[4], Mohammad-Ali Assarehzadegan[1]

1. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran 2. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Allergy and Clinical Immunology, Rasool-E-Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
3. Department of Immunology and Allergy, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Plant Biology, Faculty of Biology Science, Islamic Azad University, North Tehran Branch, Tehran, Iran

Background: Environmental pollens are known to cause exacerbation of symptoms in patients with allergic rhinitis (AR) and asthma. One of the most important causes of respiratory allergy in tropical countries is related to the Oleaceae family. During pollen months, the number of patients visiting hospitals has been shown to increase in some studies. However, in IRAN, such studies are limited. Therefore, we aimed to investigate Oleaceae airborne pollen counts and to find its correlation with the skin prick test (SPT) among patients in Alavi Zanjani Charity Asthma and Allergy Clinic in Tehran.

Methods: Aerobiological sampling was done using the Durham method for 4 years (Jan 2019 – Jan 2023). Patients coming with problems of respiratory allergy such as allergic rhinitis or asthma were recruited in the study. Skin prick tests (SPTs) were carried out after obtaining consent from these patients.

Results: Average annual Oleaceae family pollen count during 2019 and 2023 were 17634grans/cm2. In the analysis, 3 types of species or families (Oleae, Ligustrum, and Fraxinus) were identified. Aerobiological studies done in Tehran have detected that Oleae pollens have been found most frequently in May and Jun. Pollen counts for Ligustrum (Private) showed seasonal peaks during, April- May and from March for Fraxinus (Ash) pollen. October and September showed the lowest pollen counts in 4 years. Out of 148 of the patients that were skin prick tested, 45 (34.5%) subjects were chosen based on the result of the test positive SPT only for Privet, (28.3%) for olive, and (46.2%) for Ash. The correlation of the pollen counts of individual Oleaceae pollen with the SPT positivity to that pollen showed a significant correlation with the Oleaceae family only.

Conclusions: It can be concluded that there were two peaks of the pollen count in a year during March–April and August–October. Average monthly pollen counts of trees were significantly correlated with the number of hospital visits of new patients.

Keywords: Allergic rhinitis, Oleaceae, pollen count, Oleae, Ligustrum, Fraxinus

Sustained Unresponsiveness Induced by Oral Immunotherapy Is Not a Completely Symptom-Free Condition: A Prospective Case Series

Fatemeh Alizadeh[1], Morteza fallahpour[2]

1. Department of Allergy and clinical immunology. Children's medical center. Tehran University of medical science. Tehran.Iran
2. Allergy Department, Rasoul e Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

Background: Strict food avoidance is the only standard treatment for food anaphylaxis. Many attempts have been made to find alternative therapies through investigation of incidental exposure, cross-contamination, incomplete adherence, nutrients, and psychological deprivations in parallel to significant impairment of quality of life. OIT is characterized by 2 different definitions. Desensitization refers to a temporary state of unresponsiveness of the adaptive immune system to a specific antigen, which is dependent on continuous use of the predetermined amount of that food, while SU is defined as persistent unresponsiveness to that antigen, irrespective of amount and continuity of consumption. It is estimated that about 30% to 90% of individuals who undergo OIT are able to achieve desensitization.

Methods: The rate of SU is unknown, although it is reported to be between 28% and 36% in limited trials. A longer maintenance phase and increased daily use may have some role in the development of SU. In this study, 8 of 21 patients (38%) developed SU.

Results: Our study did not aim to determine the success rate of induction of SU. We attempted to provide more information about possible reactions related to milk ingestion after the achievement of SU. Our main intention was to determine whether we can really assure patients with anaphylaxis that they are completely safe from exposure to the culprit food, regardless of the dose and continuity of consumption.

Conclusions: To our knowledge, this is the first report of such patients being followed after they developed SU. However, the question of whether patients will be safe on exposure remains unanswered.

Keywords: Sustained unresponsiveness. Natural tolerance. Oral immunotherapy. Desensitization. Anaphylaxis

The effect of multi strains of probiotics on Th17-related cytokines in patients with asthma: a randomized, double-blind, placebo-controlled trial

Rasoul Baharlou[1], Sina Sadrifar[1], Tannaz Abbasi-Dokht[1], Farhad Malek[1]

1. Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran

Background: Asthma is known as one of the most common chronic inflammatory diseases characterized by recurrent obstruction and inflammation of the airways. Probiotics are defined as a group of beneficial living microorganisms that are beneficial in many disorders, including allergies. The aim of this study was to investigate the probiotic supplement effects on the improvement of clinical asthma symptoms and changes in the expression pattern of Th17-related inflammatory cytokines in asthmatic patients.

Methods: This was a randomized controlled clinical trial with parallel, double-blind groups. Forty patients with asthma were enrolled and received 1 capsule/day of a probiotic supplement for 8 weeks. Respiratory function tests; and gene expression of IL-6, IL-17, IL-21, and TGF-β were evaluated at the baseline and end of the intervention.

Results: The results showed that the gene expression of IL-6 and IL-17 in patients after receiving probiotics was reduced and expression of TGF-β was increased as compared to the baseline. Also, the expression of IL-17 and IL-21 in the probiotic group was significantly lower than the placebo group at the end of the intervention. In addition, an improvement in pulmonary function tests and clinical symptoms was observed after receiving probiotics.

Conclusion: Eight-week treatment with a probiotic supplementation suggests that it may affect on Th17 cells-associated IL-6, IL-17, and TGF-β; and Forced Expiratory Volume in 1 second and Forced Vital Capacity. Taken together, these results suggest that probiotics may have the ability to affect neutrophilic asthma and they can possibly be used besides common treatments for patients with neutrophilic asthma.

Keywords: Asthma, probiotic, randomized controlled trial, Th17

The Evaluation of Subcutaneous Immunotherapy Efficacy and Serum Vitamin D Effects in Allergic Rhinitis Adult Patients

Farahzad Jabbari-Azad[1], Maryam Joudi[2], Reza Farid Hosseini[3], Maryam Khoshkhui[1], Maryam Salehi[4], Samaneh Kouzegaran[1], Mansur Ahoon[1]

1. Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Research Center for Patient Safety, Mashhad University of Medical Sciences, Mashhad, Iran / Clinical Research Unit, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Allergic rhinitis is one of the most prevalent medical conditions, compromising the quality of life by affecting work/academic performance. While many treatment options act by inducing local effects and treating the patients symptomatically, subcutaneous allergen immunotherapy induces systemic effects and leads to the continuing resolution of allergic rhinitis problems. As the treatment responses of allergen immunotherapy might be influenced by vitamin D level, this study aimed to investigate the clinical efficacy of subcutaneous allergen immunotherapy according to the serum vitamin D level in adult patients with allergic rhinitis.

Methods: Fifty-five patients with persistent allergic rhinitis and positive skin prick test were enrolled in the present study. To assess the relationship between serum vitamin D level and the clinical efficacy of immunotherapy, the Sino-nasal Outcome Test (SNOT-22) and Mini Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ) were employed in two phases: before the immunotherapy and during the maintenance phase.

Results: After treatment, the skin prick test grade of all participants ($p < 0.001$) significantly decreased based on vitamin D level, i.e. vitamin D deficiency ($p = 0.002$), insufficiency ($p = 0.5$), suboptimal provision ($p < 0.001$), and sufficiency ($p< 0.001$). Furthermore, the greatest reduction in SNOT-22 and MiniRQLQ scores were observed in patients with vitamin D level higher than 30 ng/mL, 30-20 ng/m, 19-10 ng/mL, and lesser than 10 ng/mL ($p < 0.001$).

Conclusion: All in all, immunotherapy improved clinical symptoms and promoted the quality of life in patients with persistent allergic rhinitis, primarily in individuals with sufficient vitamin D levels.

Keywords: Allergic Rhinitis, Immunotherapy, Vitamin D

Cancer Immunology

Inhibition of apelin/APJ axis enhances the potential of dendritic cell-based vaccination to modulate Th1 and Th2 cell-related immune responses in an animal model of metastatic breast cancer

Javad Masoumi[1], Abdollah Jafarzadeh[2]

1. Tabriz University of Medical Sciences, Tabriz, Iran
2. Kerman University of Medical Sciences, Kerman, Iran

Background: Apelin as an immunosuppressor peptide is expressed in the microenvironment of many tumors. Thus, inhibition of apelin-related protumor activities can promote the effectiveness of cancer immunotherapy. Here, we investigated the efficacy of a dendritic cell (DC) vaccine in combination with an apelin receptor antagonist, ML221, to modulate Th1 and Th2 cell-related responses in breast cancer-bearing mice.

Methods: Tumor was induced in female BALB/c mice by injecting 7×105 4T1 cells in the right flank. Tumor-bearing mice were then given PBS, ML221, DC vaccine and "ML221 + DC vaccine" for 21 days. On day 37, mice were sacrificed and the frequency of Th1/Th2 cells in spleen and serum levels of IFN-γ/IL-10 were determined using flow cytometry and ELISA, respectively. Lung metastasis was evaluated in lung tissues stained with hematoxylin and eosin. Finally, the obtained data were analyzed using appropriate statistical tests.

Results: Combination therapy with ML221 + DC vaccination was more effective in reducing tumor growth ($p<0.0001$), preventing lung metastasis ($p<0.0001$) and increasing survival rate ($p<0.01$) compared to the control group. Moreover, combination treatment substantially increased the frequency of Th1 cells while decreasing the frequency of Th2 cells in the spleen compared to the control group ($p<0.01$). It also reduced serum levels of IL-10 compared with the control group ($p<0.05$).

Conclusion: Our findings showed that combination therapy using ML221 + DC vaccine can be considered as an effective cancer therapeutic program to potentiate anti-tumor immune responses.

Keywords: Breast cancer, Apelin, DC vaccine, Immunotherapy, Th1/Th2

The effect of co-treatment of metformin and PCL-sorafenib nanomedicine on MCF-7 cell line

Emad Heydarnia[1], Aref Sepasi[2], Nika Asefi[3], Javad Mohammadnejad[4]

1. Department of Medical Nanotechnology, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Medical Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Genetics, Breast Cancer Research Center, Motamed Cancer Institute
4. Department of Life Science Engineering, Faculty of New Science and Technologies, University of Tehran, Tehran, Iran

Background: Despite breakthrough therapeutics in breast cancer, it is one of the main causes of mortality among women worldwide. Thus, drug therapies for treating breast cancer have recently been developed by scientists. Metformin and Sorafenib are well-known therapeutic in breast cancer. In the present study, we combined Sorafenib and PCL-sorafenib with metformin to improve drug absorption and promote therapeutic efficiency.

Methods: The MCF-7 cells were treated with metformin, Sorafenib, or PCL-sorafenib. The growth inhibitory effect of these drugs and cell viability were assessed using MTT and flow cytometry assays, respectively. The expression of targeted genes involved in cell proliferation, signaling, and the cell cycle was measured by Real-time PCR.

Results: The results showed that MCF-7 cells treated with Metformin/sorafenib and metformin/PCL-sorafenib co-treatment contributed to 50% viability compared to untreated group. Moreover, PI and Annexin V staining tests showed that the cells viability for Metformin/sorafenib and metformin/PCL-sorafenib was 38% and 17%, respectively. Furthermore, Sorafenib and PCL-sorafenib leads to p53 expression increase by which they can increase ROS, thereby decreasing GPX4 gene expression. In addition, they affected the expression of BCL2, and BAX and altered the cell cycle.

Conclusion: To sum up, the combination of PCL-sorafenib/metformin and metformin/sorafenib increased sorafenib absorption at lower doses and also leads to apoptosis and oxidative stress increases in MCF-7 cells.

Keywords: Breast cancer, Nanoparticle, Metformin, Sorafenib, Gene expression

A New Urinary Profile of Biomarkers for Early Diagnosis of Lung Cancer

Mohammad Mahdi Mehrshad[1]

1. Zanjan University of Medical Science, Zanjan, Iran.

Background: Lung cancer is the leading major cause of cancer deaths worldwide. In this regard, one of the main challenges is that more patients are diagnosed in later stages III and IV. Of note, no clinically applied biomarkers exist yet for lung cancer screening. Furthermore, introducing a feasible and efficient strategy for a diagnosis of lung cancer could improve the outcome of the disease. It was observed that high levels of Creatine Riboside (CR) and N-acetylneuraminic acid (NANA) are relative to different types of cancer including lung cancer. In this systematic review, we assessed the capability of CR and NANA as a proper screening strategy in lung cancer.

Methods: A systemic review was conducted for relevant MESH keywords and related articles through prominent databases such as Web of Science, and PubMed, from 2014 to 2021.

Results: Our results among various studies documented that this double marker significantly increased in early stage of lung cancer (stage I) in comparison with controls. The ROC curves analysis showed the AUC (area under the curve) of CR is relatively 0.73-079 (p=0.0001), and for NANA AUC is 0.64-0.7 (p=0.002). The sensitivity and specificity of double marker combination with risk factors (such as smoking, BMI) respectively are 50%-61% and 82%-86%. Moreover, it has been shown that the level of these markers are independent of race, age, and gender.

Conclusion: Beside the promising results of CR and NANA level in the early stage of lung cancer, other advantages of these biomarkers are interesting. In this regard, the urine analyze as feasible and non-invasive tests could be performed easily on suspected patients. Moreover, the measurement assays of this metabolites are very reproducible. In addition, there are concerns related to the cost-benefit and radiation exposure risks of low-dose CT for lung cancer which indicate the priority of this new method. Accordingly, the NANA and CR have potential to be introduced as robust double biomarkers for clinical lung cancer screening.

Keywords: Lung cancer, Cancer screening, Creatine Riboside, N-Acetylneuraminic Acid"

A Systematic Review of Investigations Efficiency between CAR-T, CAR-NK, and CAR-M cells Therapy in Solid Tumors

Nastaran Nikkhou[1], Parvaneh Farzaneh[2]

1. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran

Background: In tumor immunotherapy, chimeric antigen receptor (CAR) cells, altered by genetic engineering with the human body's immune cells the therapeutic effect of expressing the target amplified and cultivated in vitro then transfused back to destroy tumor cells. The different types of CAR cell therapy according to the immunocytes into CAR-T, CAR-NK, and CAR-M cells. Solid tumors Compared to hematological tumors have the features of firm tumor heterogeneity, restricted mark antigen selection, and low T-cell infiltration to produce an immunosuppressive tumor microenvironment. These elements would show great blocks to CAR cell therapy.

Methods: The systematic databases searched were PubMed and Google Scholar All the literature was retrieved. The search query in PubMed advance search was ((("car t cell"[Title/Abstract]) OR ("car nk cell"[Title/Abstract])) OR ("car m cells"[Title/Abstract])) AND ("solid tumor"[Title/Abstract]) in had 144 results and data were included 2021 up to 2023, which was the day concluded search.

Results: CAR-NK and CAR-M cells Compared with CAR-T therapy are more effective against solid tumors and have numerous exceptional characteristics. However, the clinical efficacy of CAR-NK cells may be limited due to their brief lifespan and low cytotoxic effect on the human body. Also, CAR-M cells could present tumor antigens to T cells and activate the immune response of T cells to tumors. The limitation of cell quantity and differentiation and proliferation ability of phagocytes is far lower than the differentiation and proliferation ability of T cells and NK cells, which will seriously limit their therapeutic effect in vivo.

Conclusion: Although CAR cell therapy is just emerging in the application of solid tumors, no severe CAR cell complex serious toxicities are documented and should better solve the blocks of the immunosuppressive of solid tumors for the use of these treatment methods in further.

Keywords: CAR-T, CAR-NK, CAR-M, Solid Tumors

Alters in immune profile influence disease progression in hepatocellular carcinoma

Hossein MOtedayyen[1], Farshid Fathi[2], Reza Saidi[3]

1. Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran
2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Division of Transplant Services, Department of Surgery, SUNY Upstate Medical University Syracuse, Syracuse, NY, USA

Background: Hepatocellular carcinoma (HCC) as a chronic liver condition is largely associated with immune responses. Previous studies have revealed that different subsets of lymphocytes play fundamental roles in controlling or improving the development and outcome of solid tumors like HCC. Hence, this study aimed to investigate whether immune system changes were related to disease development in HCC patients.

Methods: Peripheral blood mononuclear cells were isolated from 30 HCC patients and 30 healthy volunteers using Ficoll density centrifugation. The isolated cells were stained with different primary antibodies and percentages of different immune cells were determined by flow cytometry.

Results: HCC patients indicated significant reductions in the numbers of CD4+ cells, Tbet+ IFNγ+ cells, and GATA+IL-4+cells in peripheral blood in comparison with healthy individuals ($p < 0.05$). There was no significant change in IL-17+RORγt+cells between patient and healthy groups. In contrast, Foxp3+CD127lowcell frequency was significantly higher in patients than healthy subjects ($p < 0.0001$). The numbers of Th1, Th2, and Th17 cells were significantly lower in HCC patients than healthy control ($p < 0.0001$), although the reduction in Th2 cell numbers was not statistically significant. On the contrary, Treg percentage showed a significant increase in patients compared to healthy subjects ($p< 0.0001$). Other data revealed that Th1, Th2, and Th17 cell frequencies were significantly higher in healthy individuals than patients with different TNM stages of HCC, with the exception of Th2 in patients with stage II HCC ($p < 0.01$-0.05). Treg percentage was significantly increased in patients with different TNM stages ($p < 0.0001$). Among all CD4+ T cells, the frequency of Th2 cell was significantly associated with TNM stages of HCC ($p < 0.05$).

Conclusion: Our data provide further evidence to show that immune changes may participate in determining HCC progression and disease outcome. However, it should be mentioned that more investigations are needed to clarify our results and explain possible impacts of other immune cells on the pathogenesis of HCC.

Keywords: Hepatocellular carcinoma, cellular immunity, T helper cells, immune system

Anticancer and Immunomodulatory activities of root and aerial part extracts and fractions of Arctium lappa L against breast cancer cell lines

Mahdieh Samei[1], Mahdieh Naghavi Alhosseini[2], Ayesheh Enayati[3], Homa Davoodi[4]

1. Cancer Research Center, Golestan University of Medical Sciences, Gorgan, Iran
2. University of Perugia, Department of Medicine and Surgery, Section of Pharmacology, 06129, Perugia, Italy
3. Ischemic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran
4. Cancer Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Background: Breast cancer is the most prevalent cancer among women in the world. Having known the problems of adverse effects of conventional cancer treatments, many studies have focused on herbal medicines because of its minimal side effects. The aim of our study was to investigate the anticancer and immunomodulatory effects of root and aerial part extracts and fractions of Arctium lappa L against breast cancer cell line.

Methods: Arctium lappa root and aerial parts were extracted with methanol, hexane, chloroform and ethyl acetate. Three breast cancer cell lines including MDA-MB-231, MCF-7 and one normal L929 cell line were considered for the study. The effects of the fractions and extracts on cytotoxicity, apoptosis and modulation of gene expression involved in TLR4, BCL-2 and BAX signaling pathways were evaluated by MTT assay, Annexin V/propidium iodide (PI) apoptosis assay, and real-time PCR, respectively. Moreover, adhesion test was carried out for all cell lines.

Results: In a dose-dependent manner, the total extract and chloroform fraction of Arctium lappa L., aerial parts showed significantly stronger effect than the roots in growth inhibition of cancer cells. Moreover, there was a significant induction of necrosis or necroptosis in cells treated with total extract and chloroform fraction of aerial parts. Real-time PCR results showed that the expression of TLR4 and NF-KB increased significantly in cancer cells treated with aerial extracts, but decreased when treated with aerial chloroform. Additionally, the ratio BAX / Bcl-2 expression reduced in cells treated with total extract and chloroform fraction of the aerial parts.

Conclusions: Since chloroform aerial part fraction showed stronger cytotoxic and gene expression modulatory effects in different cancer cell lines, it could be a potential candidate for using in future herbal medicine related treatments of breast cancer by inhibiting TLR4 signaling at certain concentrations in cases of cell resistance to chemotherapy.

Keywords: Cytotoxic, Arctium lappa L, Necroptosis, Apoptosis, TLR4, NF-KB, BAX, BCL-2, MCF-7, MDA-MB-231

Anticancer effects of Fludarabine Phosphate on colorectal carcinoma cell line (SW480)

Shiva Alipour[1], Behzad Baradaran[1]

1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Cancer, also known as malignant tumor, is a major public health problem worldwide. One of strategies for cancer immunotherapy is treatment of monocyte-derived dendritic cells with special compounds in order to induce immunogenic dendritic cells to present tumor related antigens to T cells for their activation against tumor cells, so we wanted to evaluate the effect of Fludarabine Phosphate, which is mainly used to treat chronic lymphocytic leukemia (CLL), on the expression of inflammatory and anti-inflammatory factors in monocyte-derived dendritic cells because of its STAT1 inhibitory role. But before that, the cell cytotoxicity of Fludarabine Phosphate was investigated against colorectal carcinoma cells: SW480.

Methods: This study was performed by an in vitro assay. The anti-cancer effects of Fludarabine Phosphate at different concentrations from 0.1 µM to 200 µM on SW480 cells were evaluated by MTT assay. There were no significant cytotoxic effects on the cells in any of these concentrations. According to studies, Fludarabine Phosphate is a prodrug and quickly become dephosphorylated after intravenous injection. So, that is why in the second MTT test we diluted Fludarabine Phosphate with plasma freshly isolated from human blood and treated cells in half of the wells of a 96-well plate with this solution, and the next half with Fludarabine Phosphate diluted with phosphate buffered saline (PBS) (in the second MTT test, higher concentrations of Fludarabine Phosphate were used from 85 µM to 3.5 mM).

Results: Fludarabine Phosphate using MTT assay did not show significant cytotoxicity on cells in none of our groups (diluted with plasma or with PBS).

Conclusion: No considerable cytotoxicity effect indicated the inability of Fludarabine Phosphate to impress these cells and show anticancer effects that maybe due to its being a pro-drug.

Keywords: Colorectal carcinoma, Cytotoxicity, Fludarabine Phosphate.

Anticancer effects of functionalized graphene-arginine with ginsenoside Rh2 in Balb/c mouse model with breast cancer

Shervin Dokht Farhangfar[1], Hadi Zare-Zardini[2], Farzaneh Fesahat[1]

1. Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Hematology and Oncology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Background: Ginsenoside Rh2 is a natural compound with potent anticancer activity, low bioavailability and fast plasma elimination. Efficiency and stability of this drug can be improved by nanosystems application. Moreover, using nanostructures changes the toxicity and side effects of the drug. This study aimed to evaluate gene expression, cytokines values and pathological properties of ginsenoside Rh2-containing arginine-reduced graphene in cancerous mouse model.

Methods: Thirty-two balb/c mice with breast cancer were divided into four groups of experimental I-III (6 mg/kg Rh2, 3 mg/kg Gr-Arg, and 3 mg/kg Gr-Arg-Rh2, respectively) and control group (6 mg/kg PBS). They were intravenously treated every three days for 32 days. Finally, gene expression (IL10, INF-γ, TGFβ and FOXP3), IL10 and IFN-γ values and pathological properties of tumor and normal tissues were investigated.

Results: Results showed a significant decrease of TGFβ expression in all drug treatment groups compared with control group (p =0.04). There was no significant difference among the groups in terms of other gene expression profiles and IL 10 and IFN-γ serum concentrations (p >0.05). However, the lowest concentration of IL10, as well as the highest concentration of IFN-γ in serum samples belonged to experimental III. Regarding Histopathological assessments, a severe necrosis was observed in tumor tissue of experimental III compared to other groups. The least metastasis rate and histological damage of lung tissue were also found in this group.

Conclusion: According to the results, Gr-Arg-Rh2 significantly decreased TGF-β expression and prevented from tumor progression. With regard to the histopathological assessments, this drug caused necrosis in tumor cells and well-inhibited metastasis. In addition, the lowest level of side effects was seen in group treated with Gr-Arg-Rh2. Thus, this study suggested Gr-Arg-Rh2 as a tumor inhibitor.

Keywords: Gensenoside Rh2, balb/c mouse, Graphene-Arginine, breast cancer

Astragalus Polysaccharide (APS) Mediates Immunomodulatory Effects on Crosstalk between Human Peripheral Blood Mononuclear Cells and Ovarian Cancer Cell line

Elham Shokati[1], Elahe Safari[1], Morteza Motalebnezhad[1]

1. Department of immunology, faculty of medical sciences, Iran University of Medical Sciences, Tehran, Iran.

Background: Astragalus polysaccharide (APS) as a functional component of Astragalus membranaceus has been associated with diverse biological properties such as anti-tumor and immunomodulatory activity. In the present research, the immunomodulatory effect of APS was evaluated on the proliferation of peripheral blood mononuclear cells (PBMCs), cytokine secretion, and induction of regulatory T (Treg) cells by establishing an in vitro co-culture model involving human PBMCs with A2780 human ovarian cancer (OC) cell line.

Methods: The effect of APS on the PBMCs proliferation and induction of Treg cells was evaluated through flow cytometry. Cytokine secretion was assessed by using enzyme-linked immunosorbent assay (ELISA).

Results: The results demonstrate that not only does APS significantly promote the proliferation of PBMCs ($p<0.001$), but also decreases the frequency of Treg cells ($p<0.05$). The lower secretion of interleukin (IL)-10, transforming growth factor beta (TGF-β), and vascular endothelial growth factor-A (VEGF-A) ($p<0.05$) was observed in the presence of APS, but it led to higher levels of IL-6 ($p<0.05$).

Conclusion: Since APS could potentiate the anti-tumor immune responses through improving PBMCs proliferation, declining anti-inflammatory cytokines, and reducing Treg cells frequency, it can be considered a beneficial immunomodulatory supplement in cancer therapy, especially OC.

Keywords: Astragalus polysaccharides; ovarian cancer; Inflammatory Cytokines; Regulatory T cells, Tumor microenvironment

Bioinformatics approaches in designing and verifying anti-BCMA-CAR T cells

Ali Moazzeni[1], Maryam Kheirandish[1], Gholamreza Khamisipour[2], Fatemeh Rahbarizadeh[3], Khadijeh golzadeh[1]

1. Immunology Department, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine (IBTO), Tehran, Iran
2. Department of Hematology, Faculty of Allied Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
3. Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background: Chimeric antigen receptor (CAR) cells are on the verge of becoming a powerful immunotherapeutic tool for hematologic malignancies. Designing a specific single-chain fragment variable (scFv) against tumor-associated antigens is one of the main steps of CAR designing. This study aimed to design and verify the anti-BCMA (B cell maturation antigen) CAR-T from in-silico to in-vitro.

Methods: The second generation of anti-BCMA-CAR was designed using SnapGene software. CD28 and CD3ζ were used as co-stimulatory and activation domains, respectively. Protein structure, function prediction, physicochemical complementarity at the ligand-receptor interface, and binding site analysis of the anti-BCMA-CAR construct were confirmed using different modeling and docking servers, including Expasy's ProtParam, I-TASSER, Galaxy Refine, HDock, and PyMOL software. T cells from a healthy donor were transduced with CAR construct following cloning procedures, and the PCR assay confirmed DNA integration. The expression of anti-BCMA Ag was assessed by real-time PCR and flow cytometry analysis. Relevant techniques have been used to determine CAR cells' specificity and functional activity.

Results: The in-silico study confirmed the appropriate protein folding, topology, and orientation of different domains of the Anti-BCMA-CAR construct and the precise receptor-ligand binding specificity. The in-vitro study confirmed mRNA expression of anti-BCMA construct in transduced T cells. The surface expression of Anti-BCMA-CAR was 89±1.15% on transduced T cells with ten days of persistency, at least. The expression of CD69 and CD107a as activation and cytotoxicity markers in co-culture with BCMA expressing cell line was 91.97 ± 1.7 % and 92.05 ± 1.29% in flow cytometry analysis, respectively.

Conclusion: This study has designed and verified anti-BCMA-CAR T cells with high specificity and functional activity. In-silico studies before the in-vito study are crucial in designing CAR constructs, given the most appropriate construct to less trial and error, which is more time-saving and cost-effective.

Keywords: "Immunotherapy" "chimeric antigen receptor" "bioinformatics" "in-vitro study"

Can we consider soluble herpes virus entry mediator (sHVEM) as a tumor marker?

Seyed Mohammad Javadzadeh[1], Mohsen Tehrani[1], Mohsen Keykhosravi[1], Rajeeh Mohammadian[2], Omolbanin Amjadi[5], Nasim Hafezi[1], Ehsan Zaboli[3], Mobina Montazeriun[1], Abolghasem Ajami[1]

1. Department of Immunology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran;
2. Gastrointestinal Cancer Research Center, Non-Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran
3. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran

Background: Immune checkpoint molecules have critical roles in directing immune responses into co-inhibitory and co-stimulatory signals. Herpesvirus entry mediator (HVEM) is a receptor of tumor necrosis factor receptor superfamily with unique features due to its interaction with both inhibitory and stimulatory ligands. The aim of this study was to measure the serum level of the soluble form of HVEM in patients with gastric, colorectal and breast cancers and evaluating its diagnostic and prognostic value.

Methods: The concentration of the soluble HVEM (sHVEM) was determined in the serum of 36 patients with breast cancer, 50 patients with colorectal cancer and 59 patients with gastric cancer using ELISA method. Moreover, 50 healthy donors (HD) as well as 31 patients with non-ulcer dyspepsia (NUD) were used as control groups. The patient's samples were obtained from the Biobank of Cancer Research Center, Mazandaran University of Medical Sciences, and Sari, Iran.

Results: The level of sHVEM was significantly higher in patients with gastric (p=0.006) and breast cancer ($p=0.01$) than in control groups (HD). The higher level of sHVEM was observed in colorectal cancer patients in comparison with HD group, although it was not significant. Moreover, the elevated level of sHVEM was shown to be higher significantly in stage III and IV compared to stage I and II in breast cancer (p = 0.03). Similar finding was detected in gastric and colorectal cancers, but not being statistically significant.

Conclusion: The results of the present study suggest that the serum level of sHVEM may be considered as a promising indicator for diagnosis as well as evaluating the progression of cancers such as gastric, breast and colorectal cancers.

Keywords: Herpesvirus entry mediator, gastric cancer, breast cancer, colorectal cancer

Cancer is associated with the emergence of placenta-reactive autoantibodies

Sara khorami sarvestani[1], Amir-Hassan Zarnani[2], Sorour Shojaeian[3], Ramin Sarrami-Forooshani[4], Saeed Yekaninejad[5], Kambiz Gilany[6], Abbas Ghaderi[7], Maryam hashemnejad[8], Asiie Olfatbakhsh[9], Farzane Notash Haghighat[10], Samaneh Montazeri[11], Allan Stensballe[12], Mahmood Jeddi-Tehrani[11]

1. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
2. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Biochemistry, School of Medical Sciences, Alborz University of Medical Sciences, Karaj, Iran
4. Department of ATMP, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
5. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
6. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
7. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
8. Obstetrics and Gynecology Department, School of Medical Sciences, Alborz University of Medical Sciences, Karaj, Iran.
9. Department of ATMP, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
10. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
11. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
12. Department of Health Science and Technology, Aalborg University, Aalborg, Denmark

Background: Placenta-specific antigens are minimally expressed or unexpressed in normal adult tissues, while they are widely expressed in cancer. In the course of carcinogenesis, a vast array of autoantibodies (AAbs) is produced. Here, we used a quantitative approach to determine the reactivity of AAbs in sera of patients with breast (BrC), gastric (GC), bladder (BC), and colorectal (CRC) cancers against first-trimester (FTP) and full-term placental proteome (TP) in comparison with the age- and sex-matched non-cancer individuals.

Methods: Immunohistochemistry was set to determine reactive target cells in FTP. The effect of pregnancy on the emergence of placenta reactive autoantibodies was tested using sera from pregnant women at different trimesters of pregnancy.

Results: Except for BC, patients with BrC ($p<0.0284$), GC ($p<0.0002$), and CRC ($p<0.0007$) had significantly higher levels of placenta-reactive AAbs. BrC ($p<0.0001$) and BC ($p<0.0409$) in the early stages triggered higher autoantibody reactivity against FTP. Reactivities of BrC sera with FTP did not show an association with ER, PR or HER2 expressions. Pregnancy in the third trimester was associated with the induction of TP- and not FTP-reactive autoantibodies ($P=0.0204$). The reactivity of BrCsera with placental proteins was found to be independent of gravidity or abortion. BrC sera showed a very strong and specific pattern of reactivity with scattered cells beneath the syncytiotrophoblast layer.

Conclusion: Our results showed that patients with cancer produced detectable levels of placenta-reactive AAbs, which could shed light on the future application of the placental proteome for the non-invasive early detection of cancer.

Keywords: Autoantibody, Antigen, Cancer, Placental proteins, Pregnancy

Celecoxib-treated dendritic cells-based vaccine enhanced cellular immunity protection against 4T1 tumor in a mice model

Raziyeh Zandvakili[1], Pedram Basirjafar[1], Abdollah Jafarzadeh[2]

1. Department of Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
2. Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.

Background: Prostaglandin E2 (PGE2), a cyclooxygenase product, which is produce in cancerous condition impresses dendritic cell (DC) activity and repress anti-tumor immune responses consequently. Hence, COX targeting in DC vaccine generation process, may improve DC-mediated antitumor responses. Investigation of celecoxib, a selective COX2 inhibitor, (CXB)-treated DC vaccine effects on some T cell-related parameters was our aim.

Methods: 4T1 tumor was induced in BALB/c mice, and then BC mice received DC vaccine treated with lipopolysaccharide (LPS-mDCs), LPS together with a dose 5 µM of CXB (LPS/CXB5-mDCs) and LPS together with a dose 10 µM of CXB (LPS/CXB10-mDCs). The frequency of splenic Th1 and Treg cells and amounts of IFN-γ and TGF-β production by Splenocytes, as well as, the expression of Granzyme-B, T-bet, and FOXP3 in tumors determined using flow cytometry, ELISA, and real-time PCR, respectively.

Results: Compared with the untreated tumor group (T-control), treatment with LPS/CXB10-mDCs decreased tumor growth ($p<0.0001$), escalated survival rate ($p=0.002$), increased the frequency of splenic Th1 cells ($p=0.015$), increased the IFN-γ ($p=0.006$) production by splenocytes, upregulated T-bet ($p<0.0001$) and Granzyme-B ($P=0.4485$), whereas decreased the number of Treg cells ($p=0.0219$), reduced the amounts of TGF-β production by splenocytes ($p=0.0169$), and reduced the expression of FOXP3 ($p=0.0057$) in comparison with T-control group.

Conclusions: Our findings showed that LPS/CXB-treated-DC-vaccine potently modulated antitumor immune responses in a mouse BC model.

Keywords: Breast cancer, Celecoxib, Dendritic cell vaccine, T cells, Immunotherapy

Cell-Free Supernatant from Probiotic Lactobacillus Reuteri Promotes Anti-proliferative and Anti-inflammatory Effect: A Promising Candidate in Colorectal Cancer

Amirreza Mehmandar Oskuie[1], Amirhossein Mardi[2]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Nowadays, probiotic bacteria are thought to play a role in cancer prevention and therapy, particularly via inducing apoptosis. The goal of this study is to investigate the anti-inflammatory, anti-proliferative, and pro-apoptotic effects of Lactobacillus reuteri cell-free supernatant (CFS) on colorectal cancer cell lines in vitro.

Methods: First, CFS were prepared from L. reuteri, and following the MTT test, HCT-116 and CT-26 cells were treated with determined concentrations of bacterial supernatant or with uninoculated bacterial growth medium at 24, 48, and 72 hours. Thereafter, quantitative real-time PCR (qPCR) analysis was done for investigating the relative gene expression of IL-6, c-MYC, PD-L1, and COX-2 and apoptotic genes. Additionally, flow cytometry was used to evaluate the pro-apoptotic effect of L. reuteri CSF.

Results: According to the findings of MTT and flow cytometry tests, administering CFS of L. reutri dramatically reduced the proliferation of HCT-116 and CT-26 cells and triggered apoptosis in a time- and dose-dependent manner. Furthermore, qPCR results demonstrated that exposure of CSF of L. reutri to both HCT-116 and CT-26 cells during the incubation times resulted in the downregulation of the IL-6, COX-2, and c-MYC genes in both cell lines as well as the PD-L1 gene in CT-26 cell line. In contrast, bacterial supernatant increased the expression of pro-apoptotic genes such as BAX, caspase-3, and caspase-9 in a time and dose-dependent manner.

Conclusion: In conclusion, our findings demonstrated that CSF components can be utilized in colorectal cancer as an anti-inflammatory and anti-proliferative agent. However, this study must be performed in experimental and in vivo models for further results.

Keywords: Lactobacillus reuteri, Colorectal cancer, Bacterial supernatant, Apoptosis

Chronic inflammation and its role to colorectal cancer development

Mohsen Mohammadi[1], Jalil Mehrzad[2], Nourouz Delirezh[3], Abbas Abdollahi[4]

1. PhD of Immunology, Urmia University, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Professor of Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Associated Professor of Immunology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
4. Associated Professor of Surgery, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Colorectal cancer (CRC) is the third common cancer and fourth cancer related mortality, worldwide. Chronic Inflammation is one of the underlying mechanisms leads to this cancer. The final aim of this research was to assess the expression level of pattern recognition receptor (PRRs)-related genes TLR2، TLR4، NLRP3 ،NF-kβMBL1,GAL1 and oxidative agent NOS2 in colon tissue and blood monocytes of CRC patients to find the relative role of chronic inflammation in the initiation of the CRC development.

Mathods: By this approach, the expression level of mentioned genes was assessed in colon tissues and monocytes of 24 cases (12 CRC and 12 healthy control persons) from 65±11 yrs old men by qPCR method.

Results: In CRC patients' monocytes, the expression level of TLR2, TLR4, GAL1 and MBL1 genes were significantly less than those of healthy controls ($p<0.05$). The expression level of NOS2 and NF-kβ genes in CRC patient's monocytes was significantly higher than those of in healthy controls ($p<0.05$). In cancerous colon tissues, the expression level of TLR2, TLR4 and NLRP3 was significantly higher and in the case of NOS2 and NF-kβ the difference was not significant. But, the expression level of MBL1 was significantly lower than normal tissues ($p<0.05$).

Conclusion: In conclusion, since, CRC development is normally accompanied by chronic inflammation; the observed significant differences at the expression level of some key inflammatory genes in monocytes and cancerous colon tissues of CRC patients, is highly related to chronic inflammatory reactions in tumor microenvironment.

Keywords: Toll like receptors 2, 4, NLR family pyrin domain containing 3(NLRP3), Nitric oxide synthase2 (NOS2), Mannose binding lectin1 (MBL1), Galectin1 (GAL1), Nuclear factor kappa binding, beta actin (ACTB), Colorectal cancer (CRC)

Clinical significance of TNFSF14/LIGHT and CD160 in gastric cancer and peptic ulcer dyspepsia

Mohsen keykhosravi[1], Abolghasem Ajami[1]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: Previous studies have reported elevated levels of soluble Herpes Virus Entry Mediator (sHVEM) in breast and gastric cancer patients. However, the expression patterns and clinical significance of CD160 and Tumor Necrosis Factor Ligand Superfamily Member 14 (TNFSF14 or LIGHT), which are counterparts of the HVEM molecular pathways, have remained unexplored in gastric cancer and gastric dyspepsia patients.

Methods: In this study, we obtained gastric tissue biopsies from 42 patients with non-ulcerative dyspepsia (NUD) as a control group, 43 gastric cancer patients (GC), and 48 peptic-ulcerative dyspepsia patients (PUD) who underwent endoscopic examination at the Imam Khomeini Hospital in Sari, Mazandaran, Iran. The relative expression levels of LIGHT (CD258) and CD160 mRNAs were assessed using the SYBR Green method.

Results: Among the 133 gastric specimens examined, the high expression of LIGHT exhibited a significant overexpression in gastric cancer patients ($p< 0.01$). Furthermore, gastric cancer patients with stages I&II accompanied by high expression of TNFSF14/LIGHT showed elevated expression levels of LIGHT ($p<0.05$) and CD160 ($p<0.05$). Surprisingly, we found a moderate correlation between LIGHT expression level and age (n=56, approximate $p<0.01$, correlation coefficient=0.427). Additionally, LIGHT gene expression level correlated with the relative expression of CD160 gene (n=56, approximate $p<0.01$, correlation coefficient=0.435).

Conclusion: Our findings suggest that higher LIGHT expression in patients with gastric cancer may play a crucial role in immune regulation toward gastric cancer. Targeted immunotherapy that harnesses co-stimulatory molecules like LIGHT could be a promising approach for gastric cancer treatment.

Keywords: Gastric Cancer, Dyspepsia, CD160 antigen, Tumor Necrosis Factor Ligand Superfamily Member 14

Cobalt chloride-induced hypoxia induces EMT in SKBR3 and HEK293T cell lines

Maryam sadri[1], Ali Akbar Delbandi[1], Nesa Rashidi[1], Mohammad Davoudzadeh[1], Reza Falak[1], Gholam Ali Kardar[2]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

Background: Hypoxia is a common characteristic of the tumor microenvironment (TME). Hypoxia-inducible transcription factor (HIF) is expressed in response to hypoxia and can activate downstream molecular events, such as epithelial-mesenchymal transition (EMT), invasion, and angiogenesis. In this study, Cobalt chloride (CoCl2) was used to simulate hypoxia in HEK293T and SKBR3 cells in order to investigate whether this treatment could induce hypoxia-associated EMT and cell invasion.

Methods: HEK293T and SKBR3 cells were treated with different concentrations of CoCl2 for different exposure times and their viability was analyzed. To confirm successful induction of hypoxia, the expression levels of HIF1α and VEGFA mRNA were assessed. Additionally, the expression of EMT-associated markers (Snail, E-cadherin, N-cadherin, and Vimentin) as well as invasion-related genes (MMP2 and MMP9) were measured during hypoxia.

Results: We found that the viability of CoCl2-treated cells was concentration-dependent and was not attenuated at low doses. In addition, gene expression of HIF and VEGFA (as a HIF1 target gene) was upregulated following hypoxia induction. E-cadherin expression was significantly downregulated in HEK293T cells, whereas N-cadherin and Snail expression was upregulated in both cell lines. Moreover, increased MMP expression was only observed in SKBR3 cells.

Conclusion: The findings indicate that CoCl2 can mimic hypoxia in both cell lines; however, EMT was triggered in SKBR3 cells more effectively than in HEK293T cells, and invasion was only stimulated in SKBR3 cells. Therefore, SKBR3 cancer cells can be used as an EMT model to better understand its control and manipulation mechanisms and allow us to investigate new therapeutic targets to suppress tumor metastasis.

Keywords: Cobalt chloride, Epithelial-mesenchymal transition, Hypoxia, Hypoxia inducible factor

COL10A1 and TMPRSS4 May be the Potential Diagnostic Biomarkers in Pancreatic Ductal Adenocarcinoma

Neda Shajari[1], Amin Ramezani[2], Ahmad Tahmasebi[3], Abbas Ghaderi[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran and Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran and Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Institute of Biotechnology, Shiraz University, Shiraz, Iran

Background: Pancreatic cancer (PC) is one of the highly fatal malignancies with the lowest survival rate of all major organ cancers. Pancreatic ductal adenocarcinoma (PDAC) is the most aggressive and common type of PC. In most cases, diagnosis occurs in the later stages, making it impossible to perform potentially curative resection. The outlook is better for patients who are diagnosed in the earlier stages. Therefore, it is crucial to discover potential biomarkers for identifying asymptomatic premalignant or early malignant tumors of PDAC.

Methods: Ten independent PDAC microarray datasets were retrieved from Gene Expression Omnibus (GEO) database and subjected to bioinformatics analysis for identifying differentially expressed genes (DEGs) between PDAC and normal samples. The external validation and also the interaction of the expression levels of selected genes with pathological stages in patients with PDAC were assessed using the Gene Expression Profiling Interactive Analysis (GEPIA) online tool. The correlation between selected genes expression and survival in PDAC was analyzed by UALCAN platform. Finally, using qRT-PCR method, we validated our bioinformatics findings in an independent patient cohort.

Results: After collecting and analyzing microarray data from ten independent GEO datasets, we selected three upregulated DEGs, including COL10A1, CTHRC1, and TMPRSS4 for further investigation. TCGA data in GEPIA shows that the expression levels of COL10A1, CTHRC1, and TMPRSS4 were all higher in PDAC compared to normal samples with significant correlations by the pathological disease stages. The survival analysis showed that TMPRSS4 was linked with poor survival, whereas expression levels of COL10A1 and CTHRC1 were not significantly associated with survival probability in the samples. The results of qRT-PCR on independent patient samples demonstrated that COL10A1 and TMPRSS4 were significantly overexpressed in PDAC tissues in comparison to normal pancreatic tissues, while CTHRC1 expression levels were not significantly altered in PDAC compared to normal samples.

Conclusion: Our results collectively suggest that COL10A1 and TMPRSS4 may be attractive biomarkers for the diagnosis of PDAC at the mRNA level.

Keywords: Pancreatic ductal adenocarcinoma, COL10A1, TMPRSS4, Bioinformatics

Comparison of serum TNF-αlevels in patients with malignant Laryngeal cancers with control group

Seyedeh Azra Shamsdin[1], Seyedeh Neda Shamsdin[1], Fatemeh Zare[1], Shima Hosseinizade[1]

1. Shiraz University of Medical Sciences, Shiraz, Iran

Background: About 98% of laryngeal cancers are differentiated SCC. The size of the primary tumor and involved lymph nodes, advanced age, performance status, and degree and depth of invasion are significant predictors of prognosis. Unfortunately, most patients are diagnosed at advanced stages (about 75% at stage three or four), especially in supraglottic tumors, where treatment options have a much more unfavorable effect on prognosis. Since TNF-α cytokine activates control systems involved in cell proliferation, differentiation, inflammation, death, and immune regulation. Normal levels of TNF-αis important for regulating immune responses. Due to the dual role of this cytokine in the inhibition or progression of cancer, in this study, we decided to evaluate the serum level of this cytokine in patients with laryngeal cancer compared general, with the control group.

Methods: In this case-control study, 60 patients with laryngeal cancer and 30 patients were included in the study as a control group according to the inclusion and exclusion criteria. Serum TNF-αlevel was measured by ELISA method. Also, the relationship between serum TNF-αlevels in patients based on clinical and pathological characteristics was examined.

Results: A total of 60 patients and 30 healthy individuals were studied. The mean serum TNF-α level in 60 patients was higher than the mean in 30 healthy controls, which is statistically significant. Differences in serum TNF-αlevels with different stages of breast cancer, breast cancer differentiation, age, TNM stage, involvement and tumor size of the patients were not statistically significant. The difference in TNF-α levels between men and women in the patient and control groups was not statistically significant.

Conclusion: Serum TNF-α levels in patients with laryngeal cancer are higher than the control group. On the other hand, this study showed that serum TNF-α levels have no significant relationship with the clinical and pathological features of laryngeal cancer.

Keywords: Serum, laryngeal cancer, malignant, TNF-α

Comparison of the expression level of TLR2, TLR4, NLRP3 and NOS2 genes in peripheral blood monocytes of colorectal cancer patients and healthy controls

Mohsen Mohammadi[1], Nourouz Delirezh[2], Jalil Mehrzad[3], Abbas Abdollahi[4]

1. Ph.D of Immunology, Microbiology Group, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Associate Professor, Microbiology Group, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
3. Professor, Microbiology and Immunology Group, Faculty of Veterinary Medicine, University of Tehran, Iran
4. Associate Professor, Department of Surgery, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Colorectal cancer (CRC) is the third most common cancer diagnosed and the second leading cause of cancer related death for both men and women in the United States of America(US) and also, is the third and fourth common cancer in Iranian men and women, respectively. It is curable in its early stages; we hypothesized" the inflammatory gene expression level of the peripheral monocytes of CRC patients is different from control healthy persons". Therefore, this research was done with the aim of finding of the role of inflammation in the formation of CRC to help diagnosis and treatment of CRC in its early stages on the basis of its immunopathological view.

Methods: In this case-control study, the expression level of TLR2, TLR4, NLRP3 and NOS2 genes was compared following RNA extraction and cDNA synthesis from isolated monocytes of stage II CRC patients(confirmed by TNM method and before any chemotherapy and radiotherapy n=12) versus non-CRC healthy/controls (referred for CRC screening n=12) by qPCR method. The β- actin gene was used as the reference gene in this research.

Results: In CRC patients' monocytes ,the expression levels of TLR2 and TLR4 genes were significantly less than those of healthy controls ($P<0.05$). The NLRP3 gene expression level in CRC group was slightly higher but, not significant. In contrast, the expression level of NOS2 gene in CRC group was significantly higher than that of in healthy controls ($P<0.05$).

Conclusion: On the basis of the variations of the gene expression levels of TLR2, TLR4 and NOS2 in monocytes of stage II CRC patients and the role of inflammation in its formation, it is possible using this variations as CRC prognosis and in time treatment along with other methods; though, it needs more investigations.

Keywords: Beta actin, Colorectal cancer, Nitric oxide synthase 2, NLR family pyrin domain-containing protein3 (NLRP3), Toll- like Receptor 2, Toll- like Receptor 4

Correlation between PD-1 inhibition and c-MYC expression in oral squamous cell carcinoma cell line (HN5)

Ramtin Shamir[1], Ata Moghimi[1], Leili Aghebati Maleki[2]

1. Department of Medical Laboratory Science, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Programmed death protein-1 (PD-1) and programmed death ligand protein-1 (PD-L1) are well-known immune pathways that participate in targeting expressed antigens of cancer cells. PD-1 promotes assisted self-tolerance, which is currently one of the targets of immunotherapy drugs. c-MYC is a particular transcriptional factor, regulating a wide range of cellular metabolism, consisting of growth, proliferation, and apoptosis. In this study, our goal is to find the relation between PD-L1 inhibition and c-MYC expression in apoptosis cells.

Methods: HN5 cell was transfected with PD-L1 siRNA. The viability of PD-L1 siRNA-treated and untreated HN5 cells was investigated using the MTT assay. Then, flow cytometry was used to analyze HN5 cell proliferation. Finally, qRT-PCR was used to quantify the expression of the proliferation and apoptosis -related gene c-Myc.

Results: We observed that inhibiting the PD-L1 gene has positively correlated with the expression levels of c-MYC ($p<0.0001$), which demonstrates the remarkable decrease in the expression of c-MYC and even knockdown of c-MYC decreased the amount of apoptosis.

Conclusion: Together our data demonstrated that c-MYC has an important correlation with PD-L1 expression that can serve as a target for HN-5 therapy. An understanding of this correlation would allow the evolution of new approaches to enhance antitumor immunity and develop novel treatments.

Keywords: c-MYC, PD-L1, Apoptosis, siRNA

Curcumin Increases Immune-Stimulatory Properties of human Breast Cancer-Associated Fibroblast via COX-2 Inhibition

Elnaz Jalilian[1], Ladan Langroudi[1]

1. Department of Medical Immunology, School of Medicine, Kerman University of medical sciences, Kerman, Iran

Background: Cancer-associated fibroblasts (CAFs) play an important role in tumor progression, including inflammation, immune evasion, carcinogenesis, and invasion. Several reports have indicated strong anti-inflammatory and anti-carcinogenic effects attributed to Curcumin. Here we report the effect of curcumin on the immunomodulatory properties of CAFs isolated from human breast cancer tissue.

Methods: Blood and tumor tissue samples were obtained from 12 breast cancer patients in stage II/III invasive ductal carcinoma. CAFs were extracted from tumor tissue, treated with curcumin, and co-cultured with PBMCs.

Results: Treatment with curcumin was able to decrease the expression of αSMA and COX-2 genes and the production of PGE2 in CAFs. In addition, in PBMCs co-cultured with CAFs treated with Curcumin, expression of FoxP3 and production of cytokines TGF-β IL-10, and IL-4 also decreased. Moreover, the expression T-bet increased, followed by an increase in the production of IFN-γ.

Conclusion: by reducing inflammation in the tumor micro-environment, curcumin was able to suppress the pro-tumor phenotype of CAFs and induce the anti-tumor phenotype in PBMCs. Thus, targeting CAFs as a component of the tumor microenvironment is applicable in combination therapies for the enhancement of anti-tumor therapies.

Keywords: Breast cancer, tumor microenvironment (TME), cancer-associated fibroblasts (CAFs), COX-2, PG-E2, curcumin

Curcumin synergistically enhanced the antiproliferative activity of doxorubicin in breast cancer

Mehdi Zandi[1], Faezeh Ghasemi[1]

1. Department of Biotechnology, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

Background: Curcumin is a polyphenolic compound derived from Curcumin longa L. There is growing body of data showing the antitumor effect of curcumin in different cancers; however the molecular mechanism underlying of this inhibition in breast cancer is still remained to be elucidated. Here we investigated the antitumor activity of curcumin alone or in combination with paclitaxel or doxorubicin in MCF-7 cells in monolayer cell cultures and spheroids models. Moreover, the cytotoxic activity of three different forms of curcumin (phytosomal), phospholipidated curcumin, amorphous curcumin and turmeric oleoresin were evaluated, compared to unformulated curcumin.

Methods: The antiproliferative activity of 4 different forms of curcumin was assessed in monolayer and spheroid models of MCF-7 cells. The cell cycle modulation and migratory behaviors of the cells were determined by FACS and migration assay before and after treatment with curcumin. The expression levels of CyclinD1, MMP3, MMP9, P65, P21, Nf-kB, and E-cadherin were studied by quantitative RT–PCR and/or western blot.

Results: Curcumin suppressed cell growth in MCF-7 cells at 110uM IC50 value. The median drug-effect analysis showed a slight-to-moderate synergism with CI values of 0.8. Curcumin was able to reduce the invasiveness of MCF-7, compared to control cells. Moreover, curcumin inhibited the tumor growth in MCF-7 cells, although this inhibition was more pronounced with amorphous/phospholipidated curcumin. Analysis of the sub-G1 region of cell cycle analysis revealed that the treatment with curcumin increased cell death through modulation of Wnt signalling pathways.

Conclusion: We demonstrated the antitumor activity of curcumin and its curcumin oleoresin in a breast cancer cell line, supporting further investigations on the therapeutic potential of this novel anticancer agent in in vivo models.

Keywords: Breast cancer, curcumin, anti-tumor effect, spheroid, oleoresin

CXCL16 and CXCR6, novel prognostic factors for breast cancer

Saad Asakere[1], Ali Langroudi[2], Behjat Kalantari Khandani[3], Gholamhossein Hassanshahi[4]

1. Department of medical Immunology School of medicine, Kerman University of medical science
2. Department of Medicine School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.M.D.
3. Hematology and Oncology Division Department of Internal medicine, Kerman University of Medical science
*4. Department of Microb*iology, Hematology, and Immunology, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

Background: Breast cancer is the most common neoplasm among women with an estimated 1.67 million new cases and causing 6.4% of the total mortality. The search for novel breast cancer risk assessments and the treatment agent is crucial for prevention and effective treatment. Among different novel agents, the chemokine CXCL16 and its associated receptor, CXCR6 has been introduced as a potential prognostic factor in breast cancer diagnosis. In this study, we compared the serum level of CXCL16 and the expression of CXCR6 in breast cancer patients and the control group.

Methods: 1:1 age and gender-matched case–control study was performed on 40 female breast cancer cases and 40 female controls without breast cancer. To confirm a breast cancer diagnosis, all patients had a pathological test. The controls were validated using pathological testing and clinical examinations. The concentration of CXCL16 was evaluated by Elisa. Real-time PCR was conducted to evaluate the expression of CXCR6 in the PBMC samples.

Results: The results depicted that the expression of CXCR6 and the concentration of CXCL16 increased significantly in patients compared to the control ($P<0.05$). Data also suggested that there is a direct association between CXCL16 and pathological stages of diagnosis as well as grades of breast cancer.

Conclusion: Current study showed the upregulation of serum CXCL16 and its receptor CXCR6 in breast cancer patients. Patients with higher levels of CXCL16 have been shown to have higher staging and grading of breast cancer. This finding suggested that the serum level of CXCL16 and its receptor can be used as a prognostic factor in breast cancer patients.

Keywords: Breast Neoplasm, Chemokine CXCL16, Mononuclear Leukocytes, Prognosis

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) silencing in colorectal cancer lysate-pulsed DCs enhances their maturation and activation

Farid Ghorbaninezhad[1], Javad Masoumi[1], Mohammad Bakhshivand[1], Behzad Baradaran[1]

1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Dendritic cell (DC)-based cancer immunotherapy is a promising treatment option for a wide range of cancers. The ability of DCs to present tumor antigens to T cells is critical for effective DC-based cancer immunotherapy. The expression of inhibitory immune checkpoints including CTLA-4 results in reduced DC-mediated anti-tumor immune responses in the tumor microenvironment. The purpose of this study was to investigate siRNA-mediated CTLA-4 silencing in colorectal cancer (CRC) lysate-pulsed DCs (mDCs) on DCs maturation and function.

Methods: After obtainment of peripheral blood mononuclear cells (PBMCs) by fractionation over Ficoll gradients from donors' peripheral blood, monocytes were isolated due to their adherence to polystyrene surfaces. Monocytes were differentiated into mature DCs using the granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-4, and CRC lysate. Mature DCs were then activated using lipopolysaccharide (LPS) and CTLA-4 expression was silenced in them via electroporation of CTLA-4 siRNA. Flow cytometry and qRT-PCR were utilized to assess the expression of antigen presentation-related markers and different cytokines in distinct groups of DCs, respectively.

Results: Compared with mDCs, CTLA-4 suppression significantly increased the expression of CD11c ($p\leq$ 0.01), CD86 ($p\leq$ 0.01), and CD40 ($p\leq$ 0.0001), while enhanced HLA-DR expression was not significantly different. In addition, CTLA-4 silencing resulted in increased TNF-α($p\leq$ 0.01) and diminished IL-10 ($p\leq$ 0.0001) expression in mDCs.

Conclusion: These findings show that suppression of CTLA-4 expression in CRC-pulsed DCs significantly increases their stimulatory function and maturation and can be considered a promising therapeutic option for CRC treatment.

Keywords: CRC, DCs, CTLA-4, Tumor lysate, siRNA

Effect of rIL-10 on the expression of granzyme B in B cells derived from tumor-draining lymph nodes of patients with breast cancer

Faezeh Absalan[1], Fereshteh Mehdipour[1], Houshang Rafatpanah[2], Abbas Ghaderi[3], Abdoul-Rasoul Talei[4]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: IL-10 is a multifunctional cytokine typically identified for its immune suppressing roles. Though, it plays a critical role in anti-tumor immunity. This cytokine is able to intensify IFN-γ and granzyme B production in TCD8+ cells. Also, this cytokine is now pondered as a survival, proliferation and differentiation element for B cells. IL10 receptor (IL-10R) is expressed on many cell types included B cells. Herein, we examined the effect of rIL-10 on the expression of granzyme B in B cells in breast tumor draining lymph nodes (TDLN).

Methods: To assess the production of Granzyme B in B cells, lymphocytes were stimulated with Recombinant IL-21 (50 or 25 ng/ml), anti-BCR (3.3 µg/ml) and Recombinant IL-10 (25 ng/ml, 50 ng/ml or 100 ng/ml) for 24 or 48 hours. In the last 6 hours, Brefeldin A (1 µl/ml) was added to the culture. Cells stimulated with only rIL-21 and anti-BCR were considered as controls. Cells were stained with antibodies against CD19 and Granzyme B and examined by flow cytometry.

Results: Under the influence of IL-21 and anti-BCR, B cells were able to produce granzyme B. But addition of IL-10 to the culture medium (in 24 or 48-hour culture) did not have a significant impact on the percentage of granzyme B producing B cells. Although, the addition of 100ng IL-10 in 24 hours, increased the frequency of B cells producing granzyme B from 8±6% to 12.6±11.3% (1.6 times greater). This test was repeated with lower doses of IL-21. Similar outcomes were obtained and the ratio of granzyme B-producing B cells augmented after stimulation by 100 ng/ml IL-10 (1.4 times greater).

Conclusion: This study revealed that IL-10 have an additive effect on the frequency of Granzyme B-producing B cells and underlined the needs of more studies on the effect of IL-10 on cytotoxicity function of B cells.

Keywords: IL-10, Granzyme B, IL-10 receptor, B cells, Tumor draining lymph nodes, Breast cancer

Effect of small interfering RNA (siRNA)-mediated silencing of PD-L1 on BCL-2 expression in HN5 Cell Line

Ata moghimi[1], Leili aghebati[2], Ramtin shamir[1]

1. Department of Medical Laboratory Science, Faculty of Paramedicine Tabriz University of Medical Sciences, Iran.
2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Background: One of the inhibitory receptors from the CD28 family can be referred to as PD-1, which recognizes two ligands called PD-L1 and PD-L2, the first one is expressed on APCs and many other tissue cells, and the second one is mainly on APCs that come from the bone marrow. They play a major role in regulating the immune response.BCL-2 can play a role in the expression and longevity of cells, especially cancer cells. This study aimed to measure the role of PDL-1 inhibition on BCL-2 expression.

Methods: To inhibit the expression of PDL-1, we inhibited it by using siRNA. We use several doses of siRNA 40, 60, and 80 pmol in 48 hours. Following PD-L1 siRNA transfection of HN5 cells and RT-PCR analysis for transfection efficiency, both transfected and untransfected cells experienced flow cytometry to assess apoptosis rate. The expression levels of the anti-apoptotic gene BCL2 measured using RT-PCR.

Results: RT-PCR analysis revealed a significant decrease in PD-L1 mRNA expression levels in HN5 cells after PD-L1 siRNA transfection. The flow cytometry analysis of apoptosis then demonstrated that PD-L1 siRNA could substantially increase the number of apoptotic HN5 cells. Ultimately, the RT-PCR analysis of PD-L1 siRNA-transfected HN5 cells found that the anti-apoptotic gene BCL2 was significantly downregulated.

Conclusion: We found that siRNA-mediated PD-L1 knockdown enhances apoptosis in HN5 cells by downregulating anti-apoptotic genes.

Keywords: PD-1, BCL-2, HN5, flow cytometry

Effects of BET Inhibitor JQ1 and Interleukin-6 on Breast Cancer Cells

Atefeh Sharif hoseini[1], Masoud Heshmati[1], Amin Soltani[1], Hedayatollah Shirzad[1], Morteza Sedehi[2], Babri A. Judd[3], Mohammad-Saeid Jami[4], Mahshad Entezam[5], Mahdi GhatrehSamani[1]

1. Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.
2. Department of Epidemiology and Biostatistics, School of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran.
3. Immunology Science Editors, Eden Prairie, MN, USA.
4. Department of Neurology, David Geffen School of Medicine, University of California Los Angeles (UCLA), Los Angeles, CA, USA.
5. Department of Microbiology and Immunology, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Background: Bromodomain and extra-terminal (BET) proteins are recognized acetylated lysine of histone 4 and act as scaffolds to recruit many other proteins to promoters and enhancers of active genes, especially at the super-enhancers of key genes, driving the transcription process and have been identified as potential therapeutic targets in breast cancer. However, the efficacy of BET inhibitors such as JQ1 in breast cancer therapy is impeded by IL-6 through an as-yet-defined mechanism.

Methods: we investigated the interplay between IL-6 and JQ1 in MCF-7 and MDA-MB-231 human breast cancer cells.

Results: we demonstrate that the efficacy of JQ1 on the inhibition of cell growth and apoptosis was stronger in MDA-MB-231 cells than in MCF-7 cells. Further, MCF-7 cells, but not MDA-MB-231 cells, exhibited increased expression of CXCR4 following IL-6 treatment. JQ1 significantly reduced CXCR4 surface expression in both cell lines and diminished the effects of IL-6 pre-treatment on MCF-7 cells. While IL-6 suppressed the extension of breast cancer stem cells (BCSCs) in MCF-7 cells, JQ1 impeded its inhibitory effect. In MCF-7 cells JQ1 increased the number of senescent cells in a time-dependent manner. Analysis of gene expression indicated that JQ1 and IL-6 synergistically increase SNAIL expression and decrease c-MYC expression in MCF-7 cells. So, the BET proteins are promising, novel therapeutic targets in late-stage breast cancers.

Conclusion: BET inhibitors similar to JQ1 show promise as therapeutic candidates for breast cancers, especially when triple-negative breast cancer cells are increased and/or tumor promoting factors like IL-6 exist in the tumor microenvironment.

Keywords: Breast Cancer, BET inhibitor, JQ1, IL-6, Breast cancer stem cell, CXCR4

Effects of breast cancer cells-derived exosomes on the expression pattern of endothelial to mesenchymal transition (EndMT) markers in HUVEC cell line

Niloofar Kherady[1], Nasibeh Besarati[1], Sepideh Maralbashi[1], Haleh Niktash[1], Tohid Kazemi[1]

1. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Tumor cells have the potential to induce expression of molecules correlated to the loss of endothelial features and gaining mesenchymal markers by several mechanism such as secreting tumor cell-derived exosomes. In this study, the expression of endothelial and mesenchymal markers in HUVEC cells were investigated before and after treatment with exosomes isolated from breast cancer cell line.

Methods: MDA-MB-231 and BT-474 cell lines were cultured in RPMI 1640 containing 5% exosome-free FBS under both normoxic and hypoxic conditions for 24 hours. Supernatants were harvested and exosomes were isolated by ultracentrifuge and confirmed by Transmission electron microscope (TEM). HUVEC cell line was treated with different concentration of exosomes for 24 hours and effects of them on proliferation of HUVEC was determined by MTT assay. After RNA segregation of the treated cell line with exosomes, cDNA synthesized and gene expression of endothelial markers (CD31 and VE-cadherin) and mesenchymal markers (MMP-9, MMP-2, N-cadherin, αSMA and Vimentin) measured by quantitative real time PCR.

Results: Our observation appeared that BT-474 cell line-derived exosomes down-regulated both endothelial (CD31, VEcadherin) and mesenchymal (MMP9, MMP2, Vimentin, αSMA, N-cadherin) markers under normoxia and hypoxia conditions. Even though MDA-MB-231 exosomes up-regulated both endothelial and mesenchymal markers under both conditions in HUVEC cell line.

Conclusion: Results of this study showed the potential impacts of exosomes as a mediator activating endothelial to mesenchymal transition markers and cancerous features in normal cells.

Keywords: breast cancer, exosome, endothelial to mesenchymal transition, hypoxia

Efficacy of therapies targeting TGF-βin solid tumors: a systematic review and meta-analysis of clinical trials

Hossein Rahavi[1], Reza Alizadeh-Navaei[2], Mohsen Tehrani[1]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Gastrointestinal Cancer Research Center, Non-communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran

Background: The efficacy of TGF-βblocking agents in some cancers has been demonstrated in preclinical studies. In this study, we conducted a comprehensive meta-analysis to explore the efficacy of anti-TGF-βtherapies in solid tumors.

Methods: PubMed, Embase, Cochrane Library, and Web of Science were searched from inception to February 20, 2021. Clinical trials reporting the efficacy of TGF-βblockers in solid tumors were eligible. Results of overall survival (OS), progression-free survival (PFS), time to progression (TTP), and overall response rate (ORR) with their 95% confidence intervals (95%CI) were calculated as the primary focus of the meta-analysis. Also, subgroup analyses were conducted according to the categories of TGF-β blocker alone or combined with chemotherapy or radiotherapy.

Results: A total of 24 studies with 1439 patients were included. The overall OS, PFS, TTP, and ORR were 10.5 months (95% CI 7.76–13.25), 2.54 months (95% CI 1.66–3.43), 4.69 months (95% CI 3.18–6.21), and 0.83 % (95% CI 0.82–0.85), respectively. Further subgroup analysis in patients who received TGF-βblockade combined with chemotherapy or radiotherapy showed that OS, PFS, TTP, and ORR were 10.92 months (95% CI 7.69–14.15), 4.09 months (95% CI 1.37–6.81), 6.00 months (95% CI 3.51–8.49), and 0.79 % (95% CI 0.75–0.83), respectively.

Conclusion: Collectively, TGF-βblockade combined with chemotherapy or radiotherapy showed more favorable clinical outcomes than monotherapy using TGF-βblockade. Further studies using high-quality clinical trials are required to validate these findings and propose new effective combinations with TGF-βblocking agents in the treatment of solid tumors.

Keywords: TGF-βInhibitors, Meta-Analysis, Solid tumor

Enhancement of folate-conjugated artemether nanoparticle-mediated immunomodulatory actions on triple negative breast cancer 4T1 tumor mouse model

Asiye Akbarian[1], Masoumeh Ebtekar[2], Nafiseh Pakravan[3], Zuhair Mohammad Hassan[4], Zahra Hasanpoor[5], Atefeh Alirezaee[6]

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Background: Since triple negative breast cancer (TNBC) is a health challenge due to lack of effective targeted therapies, it seems necessary to search for alternative treatments. Artemether (ARM) is an anticancer herbal drug owing immunomodulatory properties such as reducing regulatory T cells (Tregs).

Methods: Nanotechnology was used to improve ARM pharmacological properties using human serum albumin (HSA). Folate-conjugated ARM-HSA nanoparticles (F-ARM-HSA NPs) were constructed against folate receptor alpha (FRα)-overexpressing TNBC cells. Analyses, including cell cytotoxicity, apoptosis, and cellular uptake were carried out on 4T1 TNBC cells (FRα+) with all drug formulations. 4T1 tumor-bearing BALB/c mice were divided into ARM, ARM-HSA, F-ARM-HSA (100 mg/kg loaded ARM), PBS, cyclophosphamide (20 mg/kg) receiving groups. In addition to tumor volume and body weight measuring, mice spleen, and tumor samples were examined for CD25+CD4+FOXP3+ Tregs frequencies, and FOXP3 mRNA expression level using flow cytometry and real time PCR, respectively. IFN-γ, IL-10, and IL-4 protein levels in the splenic supernatants were accomplished by ELISA.

Results: The in vitro analyses on 4T1 cells showed increased cytotoxicity and cellular uptake of folate-conjugated drug. F-ARM-HSA supplementation, suppressed tumor volume and increased mice survival status. Tregs frequencies and FOXP3 mRNA level were significantly reduced after F-ARM HSA treatment. Folate-conjugated NPs administration led to a significant increase in IFN-γand a decrease in IL-10 and IL-4.

Conclusion: Our results demonstrated that ARM targeting with folate against folate receptor-expressing cancer cells potentially led to better therapeutic efficacy of ARM, such as reducing Tregs. Acceptable performance of F-ARM HSA NPs in TNBC animal model can provide promising future for TNBC treatment.

Keywords: Artemether (ARM), Folate targeting, Nanoparticles, Triple negative breast cancer (TNBC), Regulatory T cells (Tregs)

Evaluating the effect of IL-1 receptor accessory protein (IL1RAP) targeting on the expression of E-Cadherin and N-Cadherin in breast cancer cell line

Ali Zarezadeh mehrabadi[1], Nazanin Aghamohamadi[1], Reza Falak[1]

1. Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Breast cancer is one of the deadliest malignancies in the world. Recent studies demonstrated that IL-1RAcP expression is significantly increased in a variety of malignancies, including breast cancer cells. This molecule is a component of the IL-1 family cytokine receptor complex and is essential for the successful signaling of IL-1 inflammatory pathways. IL-1 can induce endothelial to mesenchymal transition (EMT) in cancer cells. EMT is one of the primary mechanisms involved in cancer cell invasion and metastasis, and it appears that IL-1RAcP plays a key role in EMT and cancer cell invasion, making it a potential therapeutic target. It seems that suppressing IL-1RAcP expression by inhibiting the inflammatory pathways involved in the EMT process can prevent breast cancer cells invasion.

Methods: Santa Cruz's siRNA transfection kit was used to transfect BT-549 cells. To assess transfection efficiency, IL-1RAcP expression was evaluated using the Real-Time PCR method. RT-PCR was also used to evaluate alterations in the expression of E-Cadherin and N-Cadherin. The Scratch wound healing assay method was used to assess cell migration in various groups of this study.

Results: IL-1βdecreased the expression of E-Cadherin and increased N-Cadherin and upregulated the EMT process in breast cancer cell line. Suppression of IL-1RAcP expression by siRNA may inhibit IL-1β-induced EMT. IL-1βincreases cell migration in the Scratch wound healing assay, and targeting the IL-1RAcP reduced the migration of BT-549 breast cancer cells.

Conclusion: The findings of this study showed that IL-1RAcP could possibly be considered as one of the molecules involved in EMT, invasion and metastasis of breast cancer cell lines, and targeting this protein could be effective in controlling the migration of breast cancer cells, especially triple-negative cell lines.

Keywords: EMT, Breast cancer, Metastasis, IL-1RAcP

Evaluation of anti-cancer effects of Newcastle virus and copper nanoparticles on breast cancer cell line (MCF-7)

Ghazaal Vakilzade[1], Shagayegh Yazdani[2], Hadi Esmaeili Gouvarchin Ghaleh[3]

1. Department of Microbiology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
2. Department of Microbiology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
3. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Background: In the entire world, cancer is one of the leading causes of morbidity and mortality and is regarded as the most significant cause of human death. One of the most prevalent malignancies in women is breast cancer, and during the past 20 years, we have seen an increase in the disease's prevalence of about 30%. Chemotherapy, one of the most popular treatments for cancer, is not without its drawbacks, including adverse effects on healthy cells. As a result, new treatment techniques were used, and efforts were made to minimize these side effects and improve remedies. This study aims to examine the anticancer effects of copper nanoparticles and Newcastle virus on the breast cancer MCF-7 cell line.

Methods: The present study was carried out in cell culture conditions. After culturing MCF-7 cells, they were treated with different concentrations of copper nanoparticles and Newcastle virus. Then, the proliferation rate of cancer cells and their apoptosis percentage were determined with MTT assay and fluorescent assay by Acridine Orange/Propidium Iodide. Significant levels were determined to be statistical differences below 0.05 ($p<0.05$).

Results: According to the findings, copper nanoparticles and the Newcastle oncolytic virus significantly reduce the growth of MCF-7 cancer cells in a dose-dependent manner. Also found that copper nanoparticles and Newcastle virus have synergistic effects in reducing proliferation and increasing apoptosis.

Conclusion: The present study's findings suggest that the aforementioned anticancer treatment effects are enhanced when nanoparticles and oncolytic viruses are used in combination and at the same time.

Keywords: Breast Cancer"MCF-7 cell line" Copper nanoparticle" Newcastle virus" Synergistic effects

Evaluation of anti-cancer effects of Newcastle virus and Gold nanoparticles on cervical cancer cell line (TC-1)

Parisa Khalaji[1], Shagayegh Yazdani[1], Mahdieh Farzanehpour[2], Hadi Esmaeili Gouvarchin Ghaleh[2], Ghazal Vakilzadeh[3]

1. Department of Microbiology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
2. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3. Department of Microbiology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.

Background: Cervical cancer is the third most common cancer in women, which has a high prevalence in developing countries. Due to proper screening programs, this cancer can be prevented. Treatment methods of radiotherapy, chemotherapy and surgery have been approved for the treatment of cervical cancer, but due to their non-selective performance in cancer treatment, researchers are looking for targeted multi-factorial treatment methods with high selective power. Therefore, the purpose of this study is to investigate the anticancer effects of Newcastle virus and gold nanoparticles on cervical cancer cell line (TC-1) separately and combined treatment with the approach of evaluating the synergistic effects of two therapeutic agents.

Methods: after culturing cervical cancer cells (TC-1 cell line), they were treated with different concentrations of gold nanoparticles (1, 2, 4, 8, 16, and 32 µg/ml) and different virus dilutions. Newcastle oncolytic (10-1 to 10-14) was used to calculate IC50 of gold nanoparticles and Titr (TCID50/ml) of Newcastle virus. In order to investigate the synergistic anticancer effects of gold nanoparticles and Newcastle oncolytic virus, TC-1 cancer cells were treated with IC50 concentration of nanoparticles and Titr (TCID50/ml) of oncolytic virus at the same time. Then the survival rate and apoptosis of TC-1 cancer cells were checked by MTT test and fluorescent assay, respectively. A statistical difference of less than 0.05 was considered as a significant level.

Results: The results of the present study showed that gold nanoparticles and Newcastle oncolytic virus cause a significant decrease in survival and a significant increase in apoptosis of TC-1 cells in a dose-dependent manner. It was also found that gold nanoparticles and Newcastle virus have synergistic effects in reducing survival and increasing apoptosis.

Conclusion: According to the results of the present study, it seems that the combined and simultaneous use of gold nanoparticles and Newcastle oncolytic virus can be considered as a multifactorial treatment method in the treatment of cervical cancer.

Keywords: cervical cancer, TC-1 cell line, gold nanoparticle, Newcastle virus

Evaluation of apoptotic effects of Nilotinib-Chitosan nanoparticles in mouse bearing breast cancer

Mina Mohamadtaheri[1], Ali Ganji[1]

1. Department of Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.

Background: The most recurrently diagnosed cancer among women is Breast cancer (BC), insofar 2.3 million new cases of BC are globally diagnosed each year. Tyrosine kinase inhibitors like Imatinib (BCR-ABL inhibitor) have been used for the treatment of tumors. Due to numerous mechanisms of resistance to imatinib which are arisen, some novel drugs were accepted to prevail its resistances, for instance nilotinib. Chitosan as a chitin derived biocompatible polymer has anticancer impacts. Importantly, using chitosan we can deliver drugs and improve drugs' efficiency.

Methods: Considering that nilotinib and Chitosan affect apoptosis, we assessed the effect of each compound separately and nilotinib encapsulated with chitosan on BAX and BCL-2 gene expression of breast cancer mouse model (4T1). Forty female BALB/c mice (6-8weeks) were accidentally grouped: negative control (Neg), positive control (Pos), nilotinib (Nil), chitosan (Cs), and Nil/Cs. 4T1 BC cells were injected into the left inguinal and abdominal mammary glands of all groups except Neg group. Treated group have taken orally 75 mg/kg Nilotinib (with or without chitosan) 3 times a week for 21 days. Instantly after mice were sacrificed, tumor and spleen were surgically extracted then BAX and BCL-2 gene expression were assessed by quantitative RT-PCR.

Results: The results exhibited that, BAX gene expression in Nil/Cs group has shown a significantly increase in spleen as compared to other groups ($p<0.05$). Additionally in tumor, a slight growth of BAX mRNA expression has seen in Nil/Cs group in compression to other groups but was not statistically significant. In agreement with the findings for BAX, BCL-2 Gene expression of Nil/Cs group significantly dropped in tumor compared to all other study groups, however that was not significant in spleen.

Conclusion: This investigation has found that there is a possibility to improve the apoptotic effects of Nilotinib by encapsulating it with chitosan, which can be helpful in tumor suppressing.

Keywords: Nilotinib, Chitosan, apaptosis, breast cancer

Evaluation of dihydrofuro [3, 4-d] pyrimidine compound on K562 tumor cell line and peripheral blood mononuclear cells (PBMCs)

Alireza Hajabbas Farshchi[1]

1. PhD Student in Clinical and Experimental immunology, Department of Clinical Immunology, School of Medicine, University of Genova ,Italy.

Background: Leukemia is a particular type of cancer characterized by the failure of cell death or disability in the differentiation of hematopoietic cells. Chronic myelogenous leukaemia (CML) is the most studied kind of this cancer. In this study, the anti-cancer effect of dihydrofuro [3, 4-d] pyrimidine compound was investigated in the human leukaemia K562 cells.

Methods: The K562 cell line was cultured by initially seeding 1×106 cells per millilitre in RPMI 1640 medium. Cell viability was investigated using trypan blue exclusion and MTT assays. Cell death in cancer and normal cells was quantified using propidium iodide (PI) and acridine-orange (AO) double staining. The one-way analysis of variance (ANOVA) and Excel software was used for data analysis.

Results: dihydrofuro [3, 4-d] pyrimidine compound had a strong fatal and concentration-dependent effect on K562 cells and caused cell death mainly through induction of apoptosis. Statistical analysis of cells under a fluorescence microscope revealed a significant difference in apoptotic cell populations between treated and untreated cells.

Conclusion: The results of this investigation clearly indicated that the dihydrofuro [3, 4-d] pyrimidine compound does have cytotoxic effects in the K562 cell line. This information revealed also that this compound may prepare a new therapeutic approach for the treatment of leukemia.

Keywords: dihydrofuro [3, 4-d] pyrimidine, apoptosis, K562 tumor, MTT, CML

Evaluation of Innate and Specific lymphocytes and related cytokines in two different types of mouse breast cancer induced by 4T1 and MC4-L2 cell lines

Reihane Rasooli-Tehrani[1], Reza Valadan[1], Hossein Asgarian-Omran[1], Abolghasem Ajami[1]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: Innate lymphoid cells are tissue-resident cells that are functionally related to T cells. They may have an effective role in constructing the tumor microenvironment, but they have been less studied. Breast cancer subtypes have different immunogenicity. 4T1 cell line induces triple-negative breast cancer with the highest immune infiltration among other subtypes, and the MC4-L2 cell line induces hormone-receptor-positive breast cancer. In this study, we have examined ILCs and T cells population, and gene expression of relative cytokines in the tumor microenvironment of 4T1 and MC4-L2 mouse breast cancer models.

Methods: 4T1 and MC4-L2 cell lines were cultured and injected subcutaneously into the mouse mammary gland. After a palpable tumor appears; tumor resection was done at two-time points of tumor growth. Enzymatic digestion was taken place, the cell suspension was prepared, and after filtration; ILCs and T cells population and their relative subgroups were examined by flow cytometry. Gene expression of cytokines was evaluated by Real-time PCR. Tumor state and lung metastasis were examined by H&E staining. Cell lines proliferation assay was done by CFSE staining.

Results: 4T1 tumor model has a greater growth rate in vivo that is confirmed with a higher proliferation rate by CFSE analysis in vitro. In both tumor models at the early stage of tumor growth, T cells frequency was notably elevated ($p<0.05$), and then by tumor progression, their frequency significantly dropped ($p<0.01$), while ILCs frequency had an increasing trend in early tumors and by tumor progression, they increased significantly ($p<0.05$). In 4T1 tumor models at the early stage, the expression of IL-10, IL-13, and IL-22 were significantly increased ($p<0.01$), and in advanced tumors, IL-10 and IL-22 were significantly decreased ($p<0.05$) but IFN-y was notably elevated ($p<0.0001$), while in MC4-L2 tumor models IL-10, IL-13, IL-22 were significantly increased ($p<0.01$) at the advanced stage. In both tumor models at the second sampling, by tumor progression, the development of invasive breast carcinoma and lung metastasis were detected.

Conclusion: Although it was thought that because ILCs are components of innate immunity they may have a role at an early stage of tumor growth but we reported their frequency by tumor progression was significantly increased and this may result in their active role in the regulation of tumor microenvironment at advanced stage of tumor growth.

Keywords: Breast cancer, innate lymphoid cells, T-CD4+, T-CD8+, Cytokines

Evaluation of mRNA expression of CD244, SAP, EAT-2, and LncRNA-GSTT1-AS1 in CD8+ T-cells in ALL and AML

Armin Akbar[1], Maryam Mohammadi[1], Hossein Asgarian-Omran[2], Ahmad Najafi[1], Reza Valadan[3], Hossein Karami[4], Amir Kahrizi[1], Mohammad Naderi[5], Ehsan Zaboli[5], Mohammad Eslami[1], Mohsen Tehrani[1]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Immunology and Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
3. Molecular and Cell-Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
4. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
5. Thalassemia Research Center (TRC), Hemoglobinopathy Institute, Mazandaran University of Medical Sciences, Sari, Mazandaran Iran.
6. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

Background: Immune-checkpoint receptors play an essential role in cancer immune evasion. This study aimed to evaluate mRNA expression of CD244, its adapter molecules, SAP and EAT-2, and LncRNA-GSTT1-AS1 in CD8+ T-cells in acute leukemia.

Methods: CD8+ T cells were isolated from the peripheral blood of 21 patients with ALL and 6 with AML.

Results: We showed that the expression of CD244, SAP, and EAT-2 were significantly lower in CD8+ T-cells from ALL patients than those from the control group ($p= 0.0225$, $p= 0.0031$, and $p= 0.0375$, respectively). Also, SAP expression was significantly lower in AML patients than in the control group ($p= 0.015$). The expression level of LncRNA-GSTT1-AS1 showed no significant differences in ALL and AML patients compared to control subjects.

Conclusions: In ALL patients, both CD244 and SAP showed reduced expression in CD8+ T cells, but the expression of SAP was much lower than CD244, which indicates a lower ratio of SAP to CD244. These results suggested an inhibitory role of CD244 in ALL.

Keywords: CD244, SAP, EAT-2, LncRNA-GSTT1-AS1, ALL, AML

Evaluation of mRNA expressions of TOX factor pathway in CD8+ T-cells in ALL and AML

Maryam Mohammadi[1], Hossein Asgarian-Omran[2], Ahmad Najafi[1], Reza Valadan[3], Armin Akbar[5], Hossein Karami[4], Mohammad Naderi[4], Ehsan Zaboli[5], Mohammad Eslami[5], Mohsen Tehrani[1,2]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Immunology and Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
3. Molecular and Cell-Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran..
4. Thalassemia Research Center (TRC), Hemoglobinopathy Institute, Mazandaran University of Medical Sciences, Sari, Mazandaran Iran.
5. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

Background: TOX and NR4A are involved in epigenetic changes and promoting the process of T-cell exhaustion. This study aimed to evaluate mRNA expression of TOX, NR4A1, NR4A2, and NR4A3 in CD8+ T-cells in acute leukemia.

Methods: Blood samples were obtained from 21 ALL and 6 AML patients and 20 healthy individuals. CD8+ T-cells were isolated using MACS. Relative gene expression was then evaluated by qRT-PCR with specific primers for TOX, NR4A1, NR4A2, and NR4A3.

Results: The expression of TOX was not significantly different in CD8+ T-cells from ALL and AML patients compared in those from the control group ($p>0.05$ and $p>0.05$, respectively); however, NR4A1 expression was significantly lower in AML group than in the control group (p=0.0006). The results also showed that the expression of NR4A2 was significantly lower in both ALL and AML patients than in the control group (P = 0.0049 and P = *0.0019*, respectively). Also, the expression of NR4A3 was significantly lower in both ALL and AML patients than in the control group (P = 0.0005 and p= 0.0055, respectively).

Conclusion: In CD8+ T-cells, the expression of NR4A2 and NR4A3 were significantly lower in ALL groups. The increased expression of TOX was not significant in any of the ALL groups.

Keywords: ALL, AML, NR4A1, NR4A2, NR4A3, TOX, CD8+ T-cells

Evaluation of Nilotinib-Chitosan anti-inflammatory effect on mouse model of breast cancer

Mina Mohamadtaheri[1], Ali Ganji[1]

1. Department of Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.

Background: Breast cancer (BC) is the most recurrently diagnosed cancer between women which its incidence has increased over the last three decades. Tyrosine kinase inhibitors like nilotinib (BCR-ABL inhibitor) have been used for the treatment of tumors. Chitosan as a biocompatible polymer derived from chitin has shown anticancer effects. Importantly, it can be used to deliver chemotherapeutic drugs while can improve the effectiveness and cytotoxicity of them.

Methods: Given the impacts of nilotinib and Chitosan on cytokine levels, we assessed the effect of each compound separately and nilotinib encapsulated with chitosan on IL-6, TNF-α, IFN-γ, and TGF-β expression of breast cancer mouse model (4T1). Forty female BALB/c mice (6-8weeks) were randomly categorized in five groups: negative control (Neg), positive control (Pos), nilotinib (Nil), chitosan (Cs), and Nil/Cs. 4T1 BC cells were injected into the left inguinal and abdominal mammary glands. Treated group have taken orally 75 mg/kg Nilotinib (with or without chitosan) 3 times a week for 21 days. Instantly after mice were sacrificed, tumor and spleen were surgically extracted and IL-6, TNF-α, IFN-γ, and TGF-β gene expression were assessed by RT-PCR.

Results: The gene expression results exhibited that, IL-6 in Nil group has a dramatically increase in spleen and tumor compared to other groups (p<0.05). An increased TNF-α mRNA expression level has seen in nil group in compression to Nil/Cs and Cs groups in tumor (p<0.05), although there were no significant differences between TNF-α of study groups in spleen. In addition, an increased IFN-γ in spleen have been demonstrated in Ni/Cs compared to other groups (p<0.05). The difference which has seen in TGF-β mRNA levels was not statistically significant between study groups.

Conclusion: This Study has found that Nil/Cs can make a contribution to elimination of Nilotinib inflammatory effects. Besides, it can be a great help in promoting anticancer immunity by elevating IFN-γ levels.

Keywords: Nilotinib, chitosan, breast cancer

Evaluation of phenotype and cytotoxic activity of natural killer cells derived from human umbilical cord blood stem cells

Farhoodeh Ghaedrahmati[1], Nafiseh Esmaeil[1]

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Natural killer (NK) cells play a crucial role in the fight against cancer as a central component of the innate immune system and many anti-cancer immunotherapy approaches have been developed by these cells. NK cells are derived from several sources and among them, umbilical cord blood (UCB) is a rich source of hematopoietic stem cells that can be differentiated in vitro into high numbers of functional NK cells. In this study, we sought to develop functional NK cells from human UCB CD34+ stem cells.

Methods: UCB samples were collected from full-term normal deliveries. The CD34+ cells were immunomagnetically selected using a magnetic-activated cell sorting (MACS). CD34+ stem cells were expanded in the presence of stem cell growth factor (SCF), thrombopoietin (TPO), interleukin (IL)-6, and fms-like tyrosine kinase 3 ligand (FLT3L), and then differentiated into the NK cells during 14 days in the presence of SCF, IL-7, FLT3L, insulin-like growth factor 1 (IGF-1), IL-15, and IL-2. The purity of the isolated CD34+ cells and differentiated NK cells was assessed by flow cytometry. Cytotoxic degranulation of NK cells (CD107a expression) against target cells (K562 cell line) and apoptosis of target cells were assessed by flow cytometry.

Results: The purity of the enriched CD34+ stem cells and CD56+CD16+ differentiated NK cells was >90%. We found that the expression of CD107a as a cytotoxic marker was significantly increased on NK cells in the 2:1 ratio of effector/target. Moreover, NK cells significantly induced apoptosis in the K562 cell line in vitro.

Conclusion: NK cells differentiated from ex vivo-expanded CD34+ stem cells provide a promising cell source for NK therapy and genetic manipulation of these cells for cancer immunotherapy.

Keywords: CD34+ stem cells, natural killer cells, umbilical cord blood, cytotoxicity

Evaluation of pyrido[3′,2′:4,5]furo[3,2-d]pyrimidine compound on K562 tumor cell line and peripheral blood mononuclear cells (PBMCs)

Alireza Hajabbas Farshchi[1]

1. PhD Student in Clinical and Experimental immunology, Department of Clinical Immunology, School of Medicine, University of Genova ,Italy.

Background: Leukemia is a particular type of cancer characterized by the failure of cell death or disability in the differentiation of hematopoietic cells. Chronic myelogenous leukaemia (CML) is the most studied kind of this cancer. In this study, the anti-cancer effect of pyrido [3′, 2′:4, 5] furo [3,2-d]pyrimidine compound was investigated in the human leukaemia K562cells.

Methods: The K562 cell line was cultured by initially seeding 1×106 cells per millilitre in RPMI 1640 medium. Cell viability was investigated using trypan blue exclusion and MTT assays. Cell death in cancer and normal cells was quantified using propidium iodide (PI) and acridine-orange (AO) double staining. The one-way analysis of variance (ANOVA) and Excel software was used for data analysis.

Results: Pyrido [3′, 2′:4, 5] furo [3,2-d]pyrimidine compound had a strong fatal and concentration-dependent effect on K562 cells and caused cell death mainly through induction of apoptosis. Statistical analysis of cells under a fluorescence microscope revealed a significant difference in apoptotic cell populations between treated and untreated cells.

Conclusion: The results of this investigation indicated that the pyrido [3′, 2′:4, 5] furo [3, 2-d] pyrimidine compound does have cytotoxic effects in the K562 cell line. This information revealed also that this compound may prepare a new therapeutic approach for the treatment of leukaemia.

Keywords: pyrido [3′, 2′:4, 5] furo [3, 2-d] pyrimidine, apoptosis, K562 tumor, CML.

Evaluation of the adenosine-treated splenocytes supernatant's effects on 4T1 cell line

Maryam Talebi Leili[1], Ahmad Ghorbani[2], Nowruz Delirezh[1], Seyyed Meysam Abtahi Froushani[1]

1. Department of Microbiology, School of veterinary, Urmia University, Urmia, Iran
2. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: Breast cancer is the most common cancer among women, and according to the World Health Organization, one out of every ten women suffers from breast cancer. The immune system plays a significant role in controlling malignant cells. One of the tumor-mediated immunosuppression mechanisms which have been considered as a potential therapeutic target is the purinergic signaling pathway. With the activation of this pathway, purine nucleoside adenosine increases in the tumor microenvironment and can potentially suppress the function of T and NK cells. In this study, we investigated the effects of the supernatant (conditioned medium) obtained from adenosine-treated splenocytes on the 4T1 breast cancer cell line.

Methods: Splenocytes were isolated from the mouse spleen under sterile conditions and transferred to RPMI 10% medium for subsequent culture. Then we added 100, 50, and 25 µM concentrations of adenosine to splenocytes. After 72 hours, the supernatant obtained from adenosine-treated splenocytes was separated and added to 4T1 cells. After 24 hours, survival, apoptosis-necrosis, proliferation, and growth rate of supernatant-treated 4T1 cells were evaluated using MTT and apoptosis-necrosis assay.

Results: The results show that adding the conditioned medium of splenocytes treated with 25µM adenosine concentration to 4T1 cells decreases the survival and growth rate along with increased apoptosis and necrosis of the 4T1 cell line.

Conclusion: It seems that low concentrations of adenosine can have beneficial effects on the anti-tumor function of immune cells, but at increased concentrations, the anti-tumor function of immune cells decreases.

Keywords: adenosine, breast cancer, immunotherapy, Splenocytes

Evaluation of the combined effects of Lactobacillus plantarum and Lactobacillus acidophilus on survival and proliferation of breast cancer cell line (MCF-7)

Sheida Janati[1], Fatemeh Faraji[2], Sara Minaeian[3], Ahmad Tavakoli[4], Majid Khoshmirsafa[5]

1. Faculty of Modern Biological Sciences and Technologies, University of Science and Culture, Tehran, Iran
2. Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
3. Antimicrobial Resistance Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
4. Research Center of Pediatric Infectious Diseases, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
5. Department of Immunology, Iran University of Medical Sciences, Tehran, Iran

Background: Need for novel preventive and curative approaches with more safety than the present one seems essential for breast cancer as the most common cancer among women. Probiotics are described as a group of live bacteria and yeasts with beneficial effects on human health. They have various effects and recently anti-cancer properties have been shown for them. Lactobacilli are a species of probiotics with antitumor activities. In this study, we aimed to assess the effects of Lactobacillus acidophilus (L. acidophilus) and Lactobacillus plantarum (L. plantarum) on the MCF-7 breast cancer cell line.

Methods: L. acidophilus and L. plantarum were cultured in de Man, Rogosa, and Sharpe (MRS) broth medium. Human breast cancer cell line (MCF-7) and the normal breast cell line (MCF10A) were cultured respectively in DMEM high glucose medium containing 10% heat-inactivated fetal calf serum (FBS)+1% penicillin/streptomycin and in the specific culture medium of MCF10A cell line+10% horse serum and 1% antibiotics. Effects of different concentrations of supernatant (5%, 10%, 20%, and 50%) and lysed bacterial pellets (1%, 5%, and 10%) on MCF-7 cells were examined by MTT [3-(4,5-dimethylthiazol-2-y-2,5-diphenyltetrazolium bromide] assay and Trypan blue exclusion test of cell viability. Also, Annexin/PI cell apoptosis assay kit was used to examine cell apoptosis and Real-Time PCR method was used to evaluate BCL-2 gene expression.

Results: 5% concentration of L. plantarum supernatant caused 50% death in MCF7 cells, while this concentration was 16% for L. acidophilus. In the investigation of the effect of the combination of the supernatants, it was found that the 10% concentration of the combination of the supernatants causes 50% death in MCF7 cells. These effects were seen in the 24-hour treatment. In the study of MCF10A, treatment with supernatant had no effect. Also, the effects caused by the treatment with the supernatant had better results compared to the bacterial pellet.

Conclusion: Based on the findings of the present study, L. plantarum alone had a better effect on MCF7 cells compared to L. acidophilus and also compared to the combination of both probiotics. Therefore, it is suggested that more studies be conducted to investigate the preventive and therapeutic effects of this probiotic in breast cancer.

Keywords: Lactobacillus acidophilus; Lactobacillus plantarum; Breast cancer; MCF-7; MCF10A

Evaluation of the cytotoxic effect of secretory mediators of activated Natural killer Cells on pancreatic cancer mouse model

Soroor Eslahi[1, 2], Marzieh Ebrahimi[2,3*], Alireza Khosravani[2], Melika Sheykhan[1,2], Hamid asadzadeh[4], Faezeh Shekari[2]

1. Biochemistry Department, Faculty of sciences and advanced Technologies in biology, University of Science and Culture, Iran
2.Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Iran
3. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Iran
4. Department of Adult Gastroenterology and Hepatology, School of Medicine University of Shahid Beheshti University of Medical Sciences

Background: Pancreatic cancer is one of the 10 cancers with the highest annual incidence with higher ability to resistance to conventional therapy. To date, natural killer (NK) cells are identified to target cancer cells directly or indirectly through secreting different cytokines. Different mediators are secreted by NK cells that among them extracellular vesicles (EV) due to their small size easily penetrate into the tumor environment to kill cancer cells. Therefore, in this study, we sought to evaluate the effect of secretory factors of NK cells to target pancreatic cancer in Mouse model.

Material and methods: NK cells were separated from MNCs by MACS and then expanded and activated by IL-15. Then the supernatant was removed and cryopreserved until use. The Pancreatic cancer induced by subcutaneous injection of 3*106 PANC1 cell in BL/NU nude mice. The tumor growth started from 5 day post cell injection. Then tumors randomly divided to three groups; one group was received 10*106 NK cells, second group was received 100 µl of supernatant (from6*106/ml) and last group received 100 µl PBS as control group, all intratumorally. The tumor size is measured daily and then mice were sacrificed according to ethical protocols, H&E staining was performed for the excised tumors.

Results: Daily measurement showed that both NK cells and their supernatant significantly reduced significantly the tumor size (supernatant received group 30.8 mm3, NK received group 61.25 mm3 vs. control group 636.54 mm3). The weight of tumor was higher in Control group compare to other two groups (control group 0.71g Vs. supernatant received group 0.05g, NK received group 0.0517 g). The pathological data demonstrated the vast necrosis in control group due the tumor growth. We did not find any changes in pathological study.

Conclusion: The present in vivo study demonstrates that mediators secreted from activated NK can induce tumor inhibition as well as NK cells in pancreatic cancer bearing mice model. However, the more study need to find the mechanism of inhibition and the effective mediator.

Keywords: NK - pancreatic cancer - secretory mediators of NK

Evaluation of the cytotoxic effects of N-pentyl-2, 7-diphenyl-3, 8a-dihydroimidazo [1,2-a] pyrimidin-3-amine on K562human tumor cell line

Alireza Hajabbas Farshchi[1]

1. PhD Student in Clinical and Experimental immunology, Department of Clinical Immunology, School of Medicine, University of Genova ,Italy.

Background: Previous studies indicated that pyrimidine derivatives possess anticancer properties. This study was set out to evaluate the effects of N-pentyl-2, 7-diphenyl-3,8a-dihydroimidazo[1,2-a]pyrimidin-3-amine as a pyrimidine derivative, on K562 human erythroleukemia cell line and peripheral blood mononuclear cells(PBMCs) as normal control cells.

Methods: The K562 cell line and PBMCs (1×106µl/well) were incubated for different times (24, 48 and 72 h) with serial logarithmic dilutions of analogue (0.10-100cells µl /ml). At the end of incubation time, the survival rate of treated cells was determined by the MTT method. To evaluate the antitumor property of the derivative, the doxorubicin antitumor drug was used as a control in this study.

Results: Our data indicated that this compound had profound cytotoxic effects on the k562 cell line in a dose-dependent manner. Interestingly, the IC50 value of this compound in k562 cells was lower in contrast with the IC50 value obtained in PBMCs.

Conclusion: As a result, this compound provides more favorable cytotoxicity against the k562 cell line with the lowest additive cytotoxicity in PBMCs.

Keywords: K562, N-pentyl-2, 7-diphenyl-3, 8a-dihydroimidazo [1, 2-a] pyrimidin-3-amine, PBMCs, MTT, IC50, doxorubicin

Evaluation of the cytotoxic effects pyrrolo [2, 3-d]pyrimidine compound on 4T1 mouse breast cells line

Alireza Hajabbas Farshchi[1]

1. PhD Student in Clinical and Experimental immunology, Department of Clinical Immunology, School of Medicine, University of Genova ,Italy.

Background: Previous studies indicated that pyrimidine derivatives possess anticancer properties. This study was set out to evaluate the effects of pyrrolo [2, 3-d] pyrimidine as pyrimidine derivatives, on mouse breast cancer (4T1) cells and peripheral blood mononuclear cells (PBMCs) as normal control cells.

Methods: Mouse breast cancer (4T1) cells and PBMCs (1×106 µl/well) were incubated with different concentrations (0.10-100cells µl /ml) of the pyrrolo [2, 3-d] pyrimidine for 24, 48 and 72 hours. The growth-inhibitory was investigated via 3 (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) assay.

Results: Our data indicated that this compound had profound cytotoxic effects on the 4T1 cell line in a dose-dependent manner. In all concentrations, cell viability decreased with respect to the control incubated cells in the absence of extract ($p<0.05$). Interestingly, the IC50 value of this compound in 4T1 cells was lower than the IC50 value in PBMCs.

Conclusion: As a result, this compound provides more favorable cytotoxicity in the 4T1 cell line with a low rate of cytotoxicity in PBMCs.

Keywords: 4T1, pyrrolo [2, 3-d] pyrimidine, PBMCs, MTT, IC50

Evaluating the effect of c-Kit L and Anti-c-Kit on the expression of E-cadherin and Vimentin involved in EMT process in MDA-MB-468 cancer cells

Nazanin Aghamohamadi[1], Ali Zarezadeh mehrabadi[1], Milad Karimi[1], Reza Falak[1]

1. Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran

Background: About 10-17% of breast cancers are classified as Triple-negative breast cancer (TNBC). TNBCs have been linked to a poor prognosis and the lack of targeted therapy. Among subtypes of TNBC, two groups, mesenchymal and mesenchymal-stem-like, express genes related to the process of epithelial-to-mesenchymal transition (EMT). C-Kit a receptor tyrosine kinase (RTK), has been observed to express in mesenchymal-stem like cells such as MDA-MB-468 cell line. Here we studied at first the effect of the presence of C-Kit on different breast cancer cell lines and the effect of C-Kit on the expression of mesenchymal markers and migration of TNBC and later the inhibitory effect of anti-C-Kit on this process in vitro analysis.

Methods: Different dosage of Anti-c-Kit and c-Kit L were applied to the cells at different times, and the appropriate dose and timing that had the best effect on EMT induction were selected. The effect of Anti-c-Kit and c-Kit L on the expression of E-cadherin and Vimentin was assessed by using a real-time PCR technique. We also used the Scratch test to measure migration.

Results: Treatment of cells with c-Kit L decreased 60% of E-cadherin expression and doubled the amount of Vimentin, and we observed an increase in migration in the scratch test. Moreover, treatment of cells with Anti-c-Kit leads to decreased 20% of Vimentin expression and an increase of 15-20% in E-cadherin expression.

Conclusion: Because TNBC does not respond to hormone therapy and HER2-suppressing drugs, and tends to be more malignant than others, the findings show that suppression of these cells by the c-Kit tyrosine kinase signaling pathway can be effective

Keywords: c-kit, epithelial-to-mesenchymal transition (EMT), migration, TNBC

Evaluation of the Effects of Curcumin and its Two Nanoforms on the Cancerous and Normal Cell Lines in the Presence of Radiation

Mohammad Taghi Bahreyni Toossi[1], Fereshteh Vaziri[1], Hamid Gholamhoseinian[1], Hossein Azimian[1], Mahmoud Reza Jaafari[2], Sajjad Sahab Negah[3], Elham Dolat[4]

1. Medical Physics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2. Pharmaceutics and Pharmaceutical Nanotechnology Verified email at mums.ac.ir
3. Neuroscience, Mashhad University of Medical Science Verified email at mums.ac.ir
4. Medical Physics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Background: The medicinal properties of herbs attract the public and the medical community's attention. Turmeric is considered a natural substance with many health effects, and curcumin, as effective component of turmeric, s known to have several usefulness for human health.

Methods: The dual effect of the curcumin in the presence of radiation as a radiosensitizer and radioprotector for tumor and normal cells, respectively, increases treatment efficacy. In this study we have investigated the effect of X-ray radiation (6 MV) and bulk curcumin, nano micelles, and nanoniosome of curcumin on two colorectal cancer cell lines and human fibroblast cells using colonogenic assay.

Results: The results of the dose-response curve displayed that the survival fraction in the presence of nanomicelles had a dual function on cancerous and normal cells due to a radiosensitivity and radioprotective effects on cancerous and normal cells, respectively. However, these effects were not shown in the presence of nanoniosome of curcumin.

Conclusion: In conclusion, curcumin is considered a radiosensitizer in tumor cells, and its radioprotection properties need more studies.

Keywords: Curcumin, Nanomicelle, Nanoniosome, Radiation

Evaluation of the effects of exosomes derived from glioblastoma associated mesenchymal stem cells on peripheral blood lymphocytes proliferation and monocytes function

Farshid Zamani[1], Farshid Yeganeh[1], Saeed Oraee Yazdani[1], Saeed Karima[1], Seyed Mahmoud Masiha Hashemi[1]

1. Shahid Beheshti University of medical science, Tehran, Iran

Background: Glioblastoma is most common malignancies associated with central nerves system. Despite traditional treatments for this cancer, the survival of patients is very poor and about one year. The role of cells and other factors existed in tumor micro environment (TME) have been noticed in the tumor grows, expansion, and recurrence. Mesenchymal stem cell (MSC) is one of the most important cells that can be found in the TME of majority of cancers. This cell is also found in the natural tissues of the body and has important physiological functions. In recent years, the role of exosomes as an important mediator of intercellular communication has drawn attention to the knowledge of these microvesicles. Recent findings have shown that cells exert many of their effects on target cells through exosomes. In this study, we investigated the effects of exosomes isolated from glioblastoma tumor MSCs and glioblastoma patients' serum on lymphocyte proliferation and activity of monocytes isolated from the blood of healthy volunteers and compared their performance with exosomes derived from Normal MSCs isolated from umbilical cord and serum of healthy people.

Methods: Blood and tumor samples were collected from glioblastoma patients who underwent surgery in Shohada'e Tajrish Hospital and were taken to the laboratory. In the next step, serum was isolated from the blood of these patients and MSC from their tumors. In this regard, as a control, serum was taken from the blood of healthy people and MSC was isolated from the umbilical cord. In the next step, exosomes were isolated from serums and supernatant of MSCs. In order to obtain lymphocytes and monocytes, blood was collected from healthy volunteers. At the end, the effect of exosomes on isolated lymphocytes and monocytes was evaluated.

Results: In this study, it was observed that exosomes isolated from MSCs of Glioblastoma patients decrease the proliferation of lymphocytes compared to the LPS group and the group receiving exosomes derived from umbilical cord MSCs. Also, the MTT test showed that the exosomes isolated from the serum of patients with glioblastoma decrease the proliferation of lymphocytes compared to the exosomes from the serum of healthy individuals. In addition, the treatment of monocytes with exosomes isolated from MSCs of glioblastoma patients caused a decrease in the production of NO by these cells. Compared with normal serum exosomes, exosomes derived from the serum of glioblastoma patients decreased NO production by monocytes. Regarding the power of phagocytosis of monocytes, it was observed that exosomes taken from MSCs of glioblastoma patients increased the percentage of yeast phagocytosis by monocytes. In addition, in this group of monocytes, the phagocytosis index was significantly reduced compared to the group receiving umbilical cord MSC exosome. Also, the exosome derived from the patients' serum decreased the phagocytosis power of monocytes compared to the exosome from the serum of healthy individuals.

Conclusion: MSC derived from glioblastoma inhibits the functions of the immune system against tumor cells through the production and secretion of exosome, and in this way facilitates the growth and expansion of the tumor.

Keywords: Glioblastoma, Mesenchymal stem cells, Exosome, Cancer associated mesenchymal stem cells, Immune system, lymphocyte, Monocyte, Immunomodulatory.

Evaluation of the effects of FLT3 Ligand on TIM-3 expression in leukemic myeloid cell line THP-1

Fatemeh Heidari[1]

1. Department of Immunology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran

Background: T-cell immunoglobin mucin-3 (TIM-3) has been identified as a surface molecule specifically for LSC in human myeloid malignancies. TIM-3+ AML LSCs secrete its ligand galectin-9 autocrinely. The aim of this study was to examine the expression of Tim3 on THP-1 cell line that it is widely accepted as a model for acute myeloid leukemia (AML) with FLT3 Ligand stimulation. FL is expressed by leukemic cells. The cytokine Fms-like tyrosine kinase 3 ligand (FL) is an essential regulator of hematopoiesis.

Methods: THP-1were cultured in RPMI 1640 supplied with 10% FBS. The gene and protein expression of TIM-3 were measured using q-RT-PCR and flow-cytometry methods in AML Cell line (THP-1), respectively.

Results: The expression of Tim3 gene in myeloid cells stimulated with FL was significantly higher than the normal control group and protein expression of Tim3 on THP-1 Cells were significantly increased.

Conclusion: According to our results, high level of FL can increase the expression of TIM-3 in THP-1 myeloid cells. The high expression of Tim3 on AML cells is associated with progression of AML disease

Keywords: FLT3 Ligand, TIM-3, AML, THP-1 Cell line

Evaluation of thiazolo [2′, 3′:1, 6] pyrido-[2,3-d]pyrimidine compound on K562 tumor cell line and peripheral blood mononuclear cells (PBMCs)

Alireza Hajabbas Farshchi[1]

1. PhD Student in Clinical and Experimental immunology, Department of Clinical Immunology, School of Medicine, University of Genova ,Italy.

Background: Leukemia is a particular type of cancer characterized by the failure of cell death or disability in the differentiation of hematopoietic cells. Chronic Myelogenous leukemia (CML) is the most studied kind of this cancer. In this study, the anti-cancer effect of thiazolo [2′, 3′:1,6]pyrido-[2,3-d]pyrimidine compound was investigated in the human leukemia K562cells.

Methods: The K562 cell line was cultured by initially seeding 1×106 cells per millilitre in RPMI 1640 medium. Cell viability was investigated using trypan blue exclusion and MTT assays. Cell death in cancer and normal cells was quantified using propidium iodide (PI) and acridine orange (AO) double staining. The one-way analysis of variance (ANOVA) and Excel software was used for data analysis.

Results: thiazolo [2′, 3′:1, 6] pyrido-[2, 3-d] pyrimidine compound had a strong fatal and concentration-dependent effect on K562 cells and caused cell death mainly through induction of apoptosis. Statistical analysis of cells under a fluorescence microscope revealed a significant difference in apoptotic cell populations between treated and untreated cells.

Conclusion: The results of this investigation clearly indicated that thiazolo [2′, 3′:1, 6] pyrido-[2,3-d]pyrimidine compound does have cytotoxic effects in the K562 cell line. This information revealed also that this compound may prepare a new therapeutic approach for the treatment of leukemia.

Keywords: thiazolo [2′, 3′:1, 6] pyrido-[2, 3-d] pyrimidine compound, apoptosis, K562 tumor, MTT, CML.

Evaluation of Transmembrane Phosphatase with TEnsin Homology expression in prostate cancer tissues

Nahid Zainodini[1], Monireh Mohsenzadegan[2], Elham Rismani[3], Maryam Abolhasani[4], Mohammad Morad Farajollahi1[5]

1. Department of Medical Biotechnology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran
3. Molecular Medicine Department, Pasteur Institute of Iran, Tehran, Iran
4. Department of Pathology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
5. Department of Medical Biotechnology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Prostate cancer is the second most prevalent cancer in men after skin cancer roughly 1,600,000 sufferers each year in the world. TPTE (Transmembrane Phosphatase with TEnsin Homology), encoding a peptide with 551 amino acids, belongs to the group of cancer-testis antigens (CTAs). The present study aimed to evaluate the importance of TPTE expression in prostate cancer tissues by producing a novel polyclonal antibody against TPTE protein.

Methods: We prepared polyclonal antibodies against peptides derived from TPTE's extracellular domains and examined 49 benign and 101 prostate cancer tissue samples immunohistochemically to determine the staining pattern and clinical significance of TPTE.

Results: The results revealed that the TPTE expression was significantly increased in patients with prostate cancer compared to benign prostatic hyperplasia (BPH) as the control group ($p<0.0001$). Furthermore, TPTE expression significantly increased with the severity and Gleason grade of the disease ($p=0.035$).

Conclusion: The results indicate that the high level of TPTE expression could be associated with poor prognosis in prostate cancer. However, further research is required to assess TPTE as a prognostic factor for prostate carcinoma.

Keywords: Cancer testis antigen, Immunohistochemistry, Prostate cancer, TPTE

Evaluation the anti-tumor responses in a mouse model colorectal cancer following administration of Lactobacillus casei

Reza Karbalaee[1], Morteza Izadi[2], Hadi Esmaeili Gouvarchingh aleh[3], Bahman Jalali Kondori[4], Ruhollah Dorostkar[5], Seyed Morteza Hosseini[6]

1. Students research committee, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. , Professor of Infectious Disease, Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
4. Baqiyatallah Research Center for Gastroenterology and Liver Diseases (BRCGL), Baqiyatallah University of Medical Sciences, Tehran, Iran
5. Department of Anatomical Sciences, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
6. Medicine, Quran and Hadith Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: Live microorganisms that, when given in sufficient quantities, boost the host's health are known as probiotics. Administration of Lactobacillus casei has been found to have favorable effects on a wide range of conditions, including psychiatric disorders, diarrhea, allergic diseases, and intestinal inflammation. According to recent research, several of them have immunoregulatory qualities that boost the host's defenses, particularly against colon cancer. This study's objective is to examine Lactobacillus casei's role in the treatment of colorectal cancer in a mouse model.

Methods: Female BALB/c mice with the left flank of their bodies injected with 5×106 CT-26 cells (a colonic carcinoma cell line) were used to imitate colorectal cancer in humans. They were given a probiotic, Lactobacillus casei, after being informed of the palpable tumor (109 CFU, daily, orally for three weeks). To assess the efficacy of the aforementioned therapies, 10 days following the final treatment, half of the mice in each group were put to death. To determine whether the medication had an impact on the mice's longevity, the other half of each group of mice was used. Significant levels were defined as statistical differences of less than 0.05.

Results: According to the study's findings, mice who received the treatment had considerably better survival curves and a slower rate of tumor growth than tumor-bearing mice who only received a single agent's treatment or animals used as negative controls. They have, they did. Additionally, it markedly boosted the amount of lactate dehydrogenase and nitric oxide produced by tumor-bearing mouse spleen cell culture. Additionally, compared to the control group, it dramatically boosted the secretion of IFN-γand decreased that of IL-4 and TGF-βin the spleen cell population.

Conclusion: The findings of the current investigation suggest that Lactobacillus casei may be useful in the treatment of colorectal cancer.

Keywords: Colorectal cancer, Lactobacillus casei, Nitric oxide, Lactate dehydrogenase

Evaluation the effect Oncolytic Echovirus 1 in the treatment of breast cancer in BALB/c mice

Fatemeh Aminiyan[1], Nahid Babaei[1], Hadi Esmaeili Gouvarchin Ghaleh[2], Gholamreza khamisipour[3], Mahdieh Farzanehpour[4]

1. Department of Molecular Cell Biology and Genetics, Bushehr Branch, Islamic Azad University, Bushehr, Iran.
2. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3. Department of Hematology, Faculty of Allied Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
4. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Background: The most frequent type of cancer among women is breast cancer. Globally, 2.3 million new cases of BC are projected to be diagnosed each year. Surgery, radiation, chemotherapy, hormone therapy, or biological therapies such oncolytic virotherapy are just a few of the many treatment options used to treat breast cancer. Treatment success rates for surgery and chemotherapy are modest. The topic of oncolytic virotherapy, in which a tumor is healed by viral infection, has great promise for developing new and more effective treatments. Echovirus 1 (EV1), a low-pathogenic human enterovirus, is an oncolytic virus that targets and kills malignant tumors with precision. The aim of the current investigation was to assess how oncolytic Echovirus 1 affected the management of breast cancer in BALB/c mice.

Methods: The right flanks of 20 female BALB/c mice (6–8 weeks old) were subcutaneously challenged with 4T1 cells. All of the animals were divided into two equal groups and started on virotherapy once they had all acquired visible tumors. The mice with tumors in the experimental groups either Echovirus 1 or PBS twice at a 1-week interval. To establish the immune response profile, one half of the mice were put to death one week following the final virotherapy. The rest of the animals were kept until they died naturally.

Results: Compared to tumor-bearing mice solely receiving PBS, the animals receiving the EV1 treatment significantly had better survival curves and slower rates of tumor progression. Nitric oxide generation and natural killer cell cytotoxicity were both dramatically increased by the EV1 virotherapy in the tumor-bearing mice's spleen cell culture. In addition, the EV1 virotherapy considerably raised the population of splenocytes' secretion of IFN-γ and decreased that of IL-4 and TGF-β relative to the splenocytes from the other groups.

Conclusion: According to the present study, it seems that the EV1 virotherapy can inhibit the proliferation of 4T1 cancer cell line in our mouse model of breast cancer

Keywords: Breast cancer, Oncolytic virus, Echovirus 1, immune response, BALB/c mice

Evaluation the role of IDO-kyn-AHR pathway in the triple negative breast cancer

Maryam Soltani asl[1]

1. University of Sao Paulo, Sao Paulo, Brazil.

Background: It is accepted that breast cancer is a serious disease across the globe. Despite all efforts in cancer immunotherapies, only a subset of patients responds properly to the treatments. Therefore, shedding light on the molecular mechanisms of resistance to immunotherapies is critical. The Aryl hydrocarbon receptor (AHR) is a role player in the cells genes expression related to the immune system and also its function on the cancer-cells still is unclear. In addition, the indoleamine-2,3-dioxygenase (IDO) enzyme supports the immune escape of cancer-cells. As a part of a huge project, here, we focused on the IDO-kyn-AHR pathway and blocking of this pathway from down and upstream in triple negative breast cancer-cells.

Methods: To disclose the function of AHR and IDO in breast cancer-cells, we used cell migration and clonogenic assays which mimics two main characteristics of cancer-cells in the in vitro models.

Results: The analysis of cell migration and clonogenic abilities revealed that AHR antagonist significantly decreases the rate of cell migration and colony-forming capacities in human and mouse triple negative breast cancer-cells. Moreover, the flow cytometry investigation disclosed that the blocking of AHR in cancer-cells did not affect IDO expression in triple negative breast cancer cells. Whereas, blocking the AHR with its antagonist as a downstream point of this pathway is more effective than the blocking of IDO with 1-Methyltryptophan as an upstream point of this pathway.

Conclusion: Blocking IDO just blocks the endogenous ligand sources for AHR while blocking the AHR not only prevents the effect of IDO endogenous products but also inhibits the effect of the exogenous ligands on AHR as a transcription factor. Our results suggest that AHR has more effect on cancer cells survival in comparison to IDO enzyme. This information might clarify why some patients have a poor response to immunotherapies.

Keywords: AHR; IDO; AHR antagonist; 1-Methyltryptophan; Triple negative breast cancer

Exhausted CD8+ T cells in acute lymphoblastic leukemia (ALL) show a progenitor phenotype

Armin Akbar[1], Hossein Asgarian-Omran[2], Ahmad Najafi[1], Reza Valadan[3], Hossein Karami[4], Mohammad Naderi[4], Ehsan Zaboli[5], Mohammad Eslami[5], Mohsen Tehrani[1,2]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Immunology and Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
3. Molecular and Cell-Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran..
4. Thalassemia Research Center (TRC), Hemoglobinopathy Institute, Mazandaran University of Medical Sciences, Sari, Mazandaran Iran.
5. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

Background: CD8+ exhausted T-cells are heterogeneous. PD-1 blockers recover progenitor exhausted T-cells, which subsequently differentiate into irresponsive terminally exhausted T-cells. Nuclear factor of activated T cells (NFAT), basic leucine zipper ATF-like transcription factor (BATF), and interferon regulatory factor 4 (IRF4) are TCR-responsive transcription factors that mediate T-cell exhaustion. They also interact with other transcription factors as they suppress T cell factor 1 (TCF-1) in terminally exhausted CD8+ T cells. TCF-1 overexpression is one of the main markers for progenitor exhausted CD8+ T-cells that play a crucial role in controlling tumor growth and responding to anti-PD-1 therapy, unlike terminally exhausted T cells. This study aimed to evaluate mRNA expression of NFATc1, IRF4, BATF, and TCF-1 in CD8+ T-cells in acute lymphoblastic leukemia.

Methods: Blood samples were obtained from 37 untreated ALL patients and 24 control subjects. Also, blood samples were taken from 8 ALL patients after 1-2 months induction therapy. CD8+ T-cells were isolated using MACS. Relative gene expression was then evaluated by qRT-PCR with specific primers for NFATc1, IRF4, BATF, and TCF-1.

Results: Expression levels of both TCF-1 and NFATc1 were higher in CD8+ T-cells from ALL patients than in those from the control group ($P = 0.006$ and $P = 0.039$, respectively), while BATF and IRF4 expressions were not significantly different between the two study groups ($p= 0.518$ and $p=0.897$, respectively). Also, gene expression of NFATc1, BATF, IRF4, and TCF-1 were not significantly different between new diagnoses and treated patients ($p= 0.261$, $p= 0.512$, $p=0.092$, and $P =0.604$, respectively).

Conclusion: Gene expression profile in CD8+ T-cells in ALL suggests a progenitor exhausted phenotype for these cells.

Keywords: Acute lymphoblastic leukemia, TCF-1, NFATc1, IRF4, BATF, and Progenitor exhausted T-cells

Exosomes derived from LPS-induced Macrophages increased the phagocytosis capacity of macrophages

Mahsa Taghavi-Farahabadi[1], Mohammad Mahmoudi[2], Seyed Mahmoud Hashemi[3], Nazanin Mojtabavi[2], Nima Rezaei[1]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: phagocytosis is one of the important mechanisms of innate immune cells for combating cancer cells. Macrophages as the most important phagocytes have plasticity and can reprogram from M1 to M2 and vice versa. M1 macrophages have strong phagocytosis capacity. So, reprogramming of these cells to M1 phenotype is one of the interesting strategies in cancer immunotherapy. Exosomes are Nano-vesicles that carry the components of the cells they originated. LPS-induced macrophages contain the mRNAs, miRNAs, and proteins that are related to the M1 phenotypes. So, we investigated the effects of exosomes derived from LPS-induced macrophages on polarization and phagocytosis capacity of macrophages.

Methods: In this study, the RAW264.7 murine macrophage cell line was obtained and maintained in DMEM supplemented with 10% heat-inactivated FBS. When cells reached 80% confluence, LPS was added to the culture media and incubated for 24 hours. After washing the cells with PBS, serum-free medium was added. After 24 hours, the culture media of the cells were collected and exosomes were extracted. The exosomes were characterized using flow cytometry, SEM, TEM and DLS. After treating of the RAW264.7 with exosomes, a suspension of heat-killed baker's yeast was prepared at 108 particles per ml in DMEM medium. The yeast suspension was added to macrophages at a ratio of 1:10 (macrophage: yeast). Macrophages were allowed for 60 minutes to phagocyte the particles at 37°C. To remove the free yeasts, the well was washed with PBS. The phagocytosis was observed using an inverted microscope.

Results: It was demonstrated that LPS induced macrophage polarization into M1 phenotype. Exosomes derived from M1 macrophages could increase the phagocytosis percentage, and phagocytosis index in macrophages.

Conclusion: Exosomes-derived from LPS-induced macrophages can be used for the reprogramming of macrophages as an important innate immune cell in TME.

Keywords: Macrophages, Exosomes, Tumor, Immunotherapy

Expression of immune check points and memory T cell marker in seminoma and dysgerminoma

Hadiseh Farahani[1], Amir Reza Dehghanian[2], Aida Khademolhosseini[3], Mohammad Reza Haghshenas[3], Nasrollah Erfani[4]

1. Department of Immunology, School of medicine, Shiraz University of Medical sciences, Shiraz, Iran
2. Pathology Department, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Shiraz Institute for Cancer Research, School of medicine, Shiraz University of Medical sciences, Shiraz, Iran
4. Department of Immunology, School of medicine, Shiraz University of Medical sciences, Shiraz, Iran

Background: Germ cell tumors (GCTs) are neoplasms derived from germ cells. Seminoma and dysgerminoma are testicular and ovarian germ cell tumors. They show a microenvironment rich in infiltrated immune cells and immune components. The expression of immune checkpoints is a vital mechanism by which tumor cells could mainly suppress antitumor immunity and it might be associated with unfavorable prognosis. CD45RO is considered as the indicator for the presence of memory T cells, reflecting the formation of efficient antitumor immunity. This study aimed to evaluate the expression of programmed death-1 (PD-1), programmed death-ligand 1 (PD-L1), and CD45RO in seminoma and dysgerminoma and to investigate their association with clinicopathological characteristics.

Methods: Immunohistochemistry was performed to detect PD-1, PD-L1, and CD45RO expression on tumor-infiltrating lymphocytes (TILs) as well as tumor cells in 33 seminoma and 30 dysgerminoma specimens. Their expression was assessed semiquantitatively using a weighted H-score, which considers both the intensity and extent of staining. Accordingly, the expression levels were graded as low (0–150) or high (160–300) respectively.

Results: PD-1 on TILs was expressed at a low level in 81.8% and 77.4%, and a high level in 12.2% and 19.4% of patients with seminoma and dysgerminoma, respectively. Similarly, the majority of seminoma (81.8%) and dysgerminoma (77.4%) patients exhibited a low PD-L1 expression on tumor cells, while any of these patients did not present PD-L1 at a high level. CD45RO expression was high in 66.7% and 90.3% of patients with seminoma and dysgerminoma. No significant association was found between the expression of PD-1, PD-L1, as well as CD45RO on TILs and tumor cells with age, tumor size, stage, tumor location, lymph node metastasis, capsular invasion, mitosis, and necrosis.

Conclusions: Frequent infiltration of CD45RO along with variable expression of PD-1 and PD-L1 on TILs and tumor cells may influence the efficacy of anti-tumor responses and subsequently immunotherapeutic strategies in seminoma and dysgerminoma.

Keywords: Seminoma, Dysgerminoma, Immunohistochemistry, PD-1, PD-L1, CD45RO

Expression of immune checkpoint receptors in acute lymphoblastic leukemia

Armin Akbar[1], Hossein Asgarian-Omran[1], Reza Valadan[2], Amir Kahrizi[3], Arash Poursheikhani[4], Hossein Karami[5], Mohammad Naderi[6], Shayan Sabeti[7], Mohammad-Mehdi Dindarloo[8], Ahmad Najafi[1], Mohsen Tehrani[1]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
2. Molecular and Cell-Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran
3. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
4. Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran
5. Thalassemia Research Center (TRC), Hemoglobinopathy Institute, Mazandaran University of Medical Sciences, Sari, Mazandaran Iran.
6. Thalassemia Research Center (TRC), Hemoglobinopathy Institute, Mazandaran University of Medical Sciences, Sari, Mazandaran Iran.
7. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
8. Department of Biostatistics and Epidemiology, Mazandaran University of Medical Sciences, Sari, Mazandaran Iran.

Background and Objective: Exhausted CD8+T-cells are defined by expression of some transcription factors and immune checkpoint receptors (ICRs), like PD-1, LAG-3, and TIGIT, which interact with their ligands on malignant cells and APCs. However, some ICRs have been reported to be expressed on both T-cells and tumor cells, including V-domain immunoglobulin suppressor of T cell activation (VISTA), Galectin-9, and T-cell immunoglobulin mucin-3 (TIM-3). In this study, we aimed to evaluate the mRNA expression of VISTA, Galectin-9, and TIM-3 on CD8+ T-cells and leukemic cells in acute lymphoblastic leukemia (ALL).

Methods: Blood samples were obtained from 25 untreated ALL patients and 25 control subjects. CD8+ T-cells were isolated using MACS. Relative gene expression was then evaluated by qRT-PCR with specific primers for VISTA, Galectin-9, and TIM-3. Also, the mRNA expression profile and clinical data of 154 ALL patients were obtained from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET).

Results: mRNA expression of Galectin-9 on CD8+ T-cells in ALL patients was significantly lower than those in the control group ($p=0.043$), while VISTA expression was not significantly different between the two study groups ($p=0.259$). Besides, TIM-3 expression was significantly higher in ALL patients than in the control group ($p<0.001$). Also, data obtained from TARGET showed that the relapse rate was not significantly different between patients with high and low expression of Galectin-9 and TIM-3 in leukemic cells ($p=0.360$ and $p=0.655$, respectively).

Conclusion: Collectively, gene expression results suggest an important role for TIM-3, but not VISTA and Gal-9, in ALL. Further studies are required to investigate the functional role of ICRs and their signaling pathways in ALL.

Keywords: "Acute lymphocytic leukemia", "TIM-3", "Galectin-9", "VISAT", "T-cell exhaustion", "immune checkpoint blockade".

Expression of PD-1 and its ligands, PDL-1 and PDL-2, in tumor draining lymph node of patients with breast cancer

Zahra Faghih[1], Zahra Mansourabadi[2], Majid Akrami[3], Maryam Fakhimi[4]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. 1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. 2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Breast Diseases Research Center, Department of Surgical Oncology, Shiraz University of Medical Sciences, Shiraz, Iran
4. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Immune checkpoint inhibitors (ICI) as novel therapeutic agents are increasingly used in cancer immunotherapy with the purpose of preventing immunosuppression in cancer by blocking the engagement of checkpoint proteins to their ligands. Programmed cell death protein 1 (PD-1), mainly expressed on T cells, is one of the widely studied checkpoints in different cancers. Here we aimed to assess the expression of this protein and its ligands PDL-1 and PDL-2 in tumor-draining lymph nodes of patients with breast cancer (BC).

Methods: A part of lymph node was obtained from 47 untreated patients with BC who were subjected to surgery. Samples were minced and mononuclear cells were then isolated using Ficoll-Hypaque gradient centrifugation. The cells were then surface-stained with specific antibodies for CD45, PD-1, PDL-1, and PDL-2 and acquired on a four-color FACSCalibur flow cytometer. Data were analyzed by FlowJo software.

Results: The mean frequency of total CD45+ cells in draining lymph nodes was 96.26±5.0. The surface expression of PD-1 was determined to be 11.04±8.5, while PDL-1 and PDL-2 were expressed at 1.75±0.89 and 1.7±0.85, respectively. Statistical analysis showed that the frequency of PD-1+ and PDL-2+ lymphocytes was higher in the patients with poor-grade tumors than those with moderate- and well-grade ($p=0.005$). The cells with high expression of PD-1 were more frequent in the patients with higher T-stages compared to those with lower T-stage ($p=0.026$). On the other hand, the PDL-2+ cells were more in N1 patients compared to N2-N3 groups ($p<0.05$).

Conclusion: Based on the association of PD-1 expression on CD45+ lymphocytes in draining lymph nodes of patients with BC with poor prognosis (i.e. higher T-stage and poor histological grade) an inhibitory role for this molecule could be assumed in the setting of BC. However, more assessments with a diverse panel of functional markers is needed to be studied.

Keywords: Bladder cancer, Immune checkpoint, PD-1, PDL-1, PDL-2

Expression of TIM-3 inhibitory immune checkpoint in tumor tissues of patients suffering from Bladder cancer

Farnaz Farahmand[1], Zahra Faghih[2], Ebrahim Eftekhar[3], Amir Reza Dehghanian[4], Ali Ariafar[5], Fatemeh Khajeh[6], Simin Torabinejad[7]

1. Department of Biochemistry, School of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
4. Trauma Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
5. Urology-Oncology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
6. Pathology Department, Fasa University of Medical Sciences, Fasa, Iran
7. Pathology Department, Shiraz University of Medical Sciences, Shiraz, Iran

Background: T cell immunoglobulin and mucin domain 3 (TIM-3) is one of the inhibitory checkpoints that inhibit and regulate immune responses. Its increased expression generally reflects the exhaustion of immune cells. However, limited studies were available regarding the role of TIM-3 in the pathology and prognosis of urological malignancies. Therefore, in the present study, the expression of TIM-3 in tumor tissue sections obtained from patients with bladder cancer (BC) was evaluated and its relationship with clinical and pathological parameters of the disease was assessed.

Methods: 155 patients with BC who underwent surgery were included. Five micrometers of tissue sections were prepared from formalin and paraffin-embedded blocks of their tumor tissues and immunohistochemistry was done for TIM-3 marker. The stained slides were evaluated by an expert pathologist and the frequency of tumor cells expressing this marker was separately reported in different areas (invasive margins and center of tumors). Mann-Whitney U and Chi-Square statistical tests were used for data analysis.

Results: The frequency of TIM-3+ tumor cells in BC tissues widely varies among patients (0-45%). Statistical analysis indicated that the frequency of TIM-3+ tumor cells significantly increased in the patients with higher TNM-stage, T-stage, muscle invasion, and necrosis ($p<0.05$).

Conclusion: Our findings indicated an increase in the expression of TIM-3 on the tumor cells along with tumor progression. It seems that the inhibitory microenvironments provided by tumors, partly through TIM-3 expression, suppress antitumor activities of immune cells. These results introduce TIM-3 as a potent candidate for further functional assessment and/or immunotherapies of patients with bladder cancer.

Keywords: Bladder cancer, Immune checkpoints, TIM-3, Immunohistochemistry

Frequency of T cell subsets with regulatory phenotypes in patients with acute myeloid leukemia (AML)

Zahra Faghih[1], Maral Mokhtari[2], Zahra Mansourabadi[3], Zohreh Bagheri[4], Seyedeh Neda Moosavi[5]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. 1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. 2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
5. Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: T regulatory cells (Treg) emerge as a highly heterogeneous subset of CD4+ T cells, which are generally responsible for commencement and progression of cancer by modulating the immune system. Based on relative expression of FOXP3 and CD127, these cells are subdivided into regulatory and memory subgroups. Here, we proposed to investigate the frequency of these subgroups in patients with acute myeloid leukemia (AML) as the most common leukemia in adults in comparison to those from healthy donors.

Methods: Fresh heparinized blood sample was obtained from 30 newly diagnosed patients with AML and 15 healthy donors, and were directly stained with specific antibodies for CD4, CD127, CD25, and for FOXP3 marker after fixation and permeabilization. The cells were then acquired on a four-color FACSCalibur flow cytometer and the data were analyzed by FlowJo software version 10.8.1.

Results: Our results indicated that the frequency of CD4+ lymphocytes was significantly decreased in the patients compared to the controls ($p<0.001$). Analysis also revealed that in average, 3.23% of CD4+ lymphocytes (0.21-13.10) in patients with AML had Treg phenotype (CD4+CD25+FOXP3+CD127Low/neg), while in the controls, their frequency was much lower (1.05 ± 0.90) (p=0.006). In addition, the percentages of memory Treg cells (CD4+CD25+FoxP3+CD127+) were significantly higher among the case group compared to the controls ($P<0.001$).

Conclusion: Collectively, the more prevalence of Treg cells and related subgroups including memory Treg cells in patients with AML is indicative of disease progression with the great importance of forecasting the therapeutic outcomes in these patients. However, the roles and functions of Tregs need to be further elucidated.

Keywords: Acute myeloid leukemia, Regulatory T cells, Memory Treg cells

Galectin-9 increases expression of IL-1βand its protein level in U937 cells

Nafiseh Behranvand[1], Farzad Nasri[2], Maryam Vakili moghaddam[2], Nafiseh Esmaeil[1], Elaheh Safari[1], Reza Falak[1]

1. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran
2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences

Background: Galectin-9 (Gal-9) is highly produced by myeloid leukemia stem cells and previous studies have shown its capability in provoking inflammation process in leukemia. NF-κB is a key inflammatory transcription factor, which is activated following binding of Gal-9 to its receptor (TIM-3) and is influential in expression level of multiple inflammatory factors. In this study we determined the impact of Gal-9 on IL-1β as a vital inflammatory cytokine and determined its effectiveness in proliferation and therapy-resistance of U937 as an acute myeloid leukemia (AML) cell line.

Methods: CCK-8 kit was applied to evaluate the impact of Gal-9 on proliferation of U937 cells. The effects of Gal-9 on IL-1β gene expression was determined by real-time PCR and the IL-1β protein level was measured by ELISA. Also, we assessed the effect of Gal-9 on NF-κB pathway and its phosphorylation rate. Findings were analyzed by Graph Pad Prism.

Results: In a dose-dependent manner, Gal-9 was capable to expand U937 cells ($p<0.01$). Following treatment of U937 cells with Gal-9, IL-1β gene expression was upregulated ($p<0.01$) and the protein level of IL-1βwas subsequently increased ($p<0.0001$). Moreover, we showed that Gal-9 can induce NF-κB phosphorylation ($p<0.01$).

Conclusion: We found that Gal-9 might be able to increase IL-1βsecretion as a vital inflammatory cytokine in AML and also directly increase cell proliferation. IL-1βis influential in proliferation of cancer cells and their resistance to therapy. So, targeting this critical interplay molecule is promising in AML therapy.

Keywords: Keywords: Acute myeloid leukemia; Galectin-9; Inflammation; IL-1β NF-κB.

Generation of Anti CD38 Chimeric Antigen Receptor Natural Killer Cells as an Off-the-Shelf Cancer Immune Therapy Approach

Maryam Asadi[1]

1. Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.

Background: CD38 is highly expressed on Multiple Myeloma (MM) Cells. Recently, it has been widely used as an effective target for MM therapeutic purposes. CD38 is also an important prognostic marker in diffuse large B-cell lymphoma and chronic lymphocytic leukemia. Since the highly effective CAR T cell therapy has some undesired limitations and side effects, CAR NK cells emerge as an off-the-shelf approach.

Methods: In this study, we designed humanized anti-CD38 CAR-NK cells in silico. Cells retrovirally transduced with CD38 construct and expanded, and their activity against CD38-positive cell lines was tested in vitro.

Results: The in silico analysis showed that our designed CD38-chimeric antigen receptor binds appropriately to the CD38 proteins. Then the in vitro tests implicated CD38-chimeric antigen receptor-transduced NK92 cells have significantly increased amount of IFN-γ, perforin, and granzyme secretion compared to control, and effectively lysed malignant cell lines in a CD38-dependent manner.

Conclusion: These results predicate the potential application of CD38-chimeric antigen receptor-transduced NK cells as the off-the-shelf immunotherapy for CD38-positive malignancies treatment.

Keywords: CD38, Chimeric antigen receptor Natural killer cells (CAR-NK cells), malignancy, immunotherapy

High expression of PD-1 on memory T cells in breast cancer draining lymph nodes: exhaustion or activation?

Roqayeh Noori[1], Ali Farrokhi[2], Razie Kiani[3], Abdol-Rasoul Talei[4], Reza Rasolmali[5], Zahra Faghih[6], Nasrollah Erfani[7]

1. Department of Immunology, School of medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Surgery, University of British Columbia, Vancouver, Canada.
3. Shiraz Institute for Cancer Research, School of medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Breast Disease Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
5. Department of Pathology, Shiraz Central Hospital, Shiraz, Iran
6. Shiraz Institute for Cancer Research, School of medicine, Shiraz University of Medical Sciences, Shiraz, Iran
7. Department of Immunology, School of medicine, Shiraz University of Medical Sciences, Shiraz, Iran- Shiraz Institute for Cancer Research, School of medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: It is well-documented that memory T cells play a crucial role in tumor immunity. To investigate cytotoxic memory cells in tumor-draining lymph nodes of patients with breast cancer (BC), we evaluated the expression of the PD-1 immune checkpoint molecule and its ligands, PD-L1, and PD-L2, on different subsets of CD8+ memory T cells including T memory stem cells (TSCM), central memory T cells (TCM), and effector memory T cells (TEM).

Methods: Mononucleated cells were isolated and surface-stained with appropriate fluorescent conjugated antibodies against CD4, CD8, CD45RO, CCR7, CD95, PD-1, PD-L1, and PD-L2 markers. FlowJo software package, version 10, was used to analyze the flow cytometric data acquired on a FACSAria III flow cytometer.

Results: Our analysis indicated that, on average, 49.20% (17.35-92.35) of cytotoxic T lymphocytes in draining lymph nodes of patients with BC express memory markers (CD45RO+CD95+). The TCM subset showed the highest frequency (37.41±14.97%), while the TSCM subset was the lowest (3.87%±2.53%). Assessing the PD-1/ PD-L expression on the surface of different memory CD8+ T cell subsets revealed that the expression of PD-1 is relatively low on the TSCM subset (2.78±3.54%). On the other hand, significantly higher expression of PD-1 was observed on TCM (44.75±13.44%) and TEM (65.74±11.17%) subsets. Analysis indicated that PD-L1 and PD-L2 are expressed at a low level (on average, 0.2-1.5%) on T cell subsets. No significant association was found between PD-1/ PD-L expression and the clinicopathological characteristics of the patients.

Conclusion: Our results indicated a high expression level of PD-1 on TEM and TCM, while an extremely low expression level on TSCM. More functional studies are needed to elucidate the regulatory role of the PD-1 molecule in memory cells' development and function.

Keywords: Breast cancer, Memory T cell, PD-1, PD-L1, PD-L2

Hsp70 interferes with IL-15 and PD-1 blocker-mediated NK cells cytotoxicity induction in relapsed acute myeloid Leukemia (AML) Patients

Javad Firouzi[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan

Background: Natural killer (NK) cells are critical immune cells for acute myeloid leukemia (AML) targeting. However, little is known about the relationship between using checkpoint inhibitors and Hsp70 as NK cell activators to control AML. Therefore, the study aims to find the best formulation to activate NK cells in AML patients.

Materials and Methods: In this experimental study we aimed to investigate the antitumor effects of activated NK cells pre-treated with ex-vivo Hsp70, human PD-1 (Programmed cell death protein 1) blocker, and interleukin 15 (IL-15) against AML. The NK cells were isolated from Mononuclear cells (MNCs) by using magnetic activation cell sorting (MACS) and were activated using the different combinations of Hsp70, PD-1 blocker, and IL-15 and then followed by immunophenotyping, functional assays to estimate their killing potential, and evaluation of expression pattern of PRF1, PIK3CB, PD-1, AKT-1, FAS-L, TRAIL, and GER A & B.

Results: The expression of PD-1 was significantly ($p<0.05$) reduced after NK cell activation by the different formulas of IL-15, Hsp70, and PD-1 blocker. The expression of NKG2A in the treated NK cells was reduced particularly in the IL-15 ($p<0.01$) and IL-15 + PD-1 blocker ($p<0.05$) groups. The addition of Hsp70 increased its expression. The cytotoxic effect of NK cells increased in all groups, especially in IL-15 + PD-1 blocker besides increasing interferon-gamma (IFN-γ), Granzymes, and perforin expression ($p<0.05$). All IL-15 + PD-1 blocker group changes were associated with the up-regulation of PIK3CB and AKT-1 as key factors of NK cell activation. The presence of Hsp70 reduced IFN-γreleasing, and down-regulation of PIK3CB, AKT-1, Granzymes, and perforin ($p<0.05$).

Conclusion: We suggested the combination of IL-15 and PD-1 blocker could enhance the killing potential of AML-NK cells. Moreover, Hsp70 in combination with IL-15 and PD-1 blocker interferes activation of AML-NK cells through unknown mechanisms.

Keywords: AML, NK cells, PD-1, Hsp70, Immunotherapy

IL-21 and anti-BCR activated B cells induced apoptosis in breast cancer cell line

Hosein Hakimi[1], Fereshteh Mehdipour[2], Morteza Samadi[1]

1. Department of Immunology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: B cells play different and sometimes opposing roles in immunity against cancer. Granzyme B (GrB)-producing B cells can directly kill tumor or effector T cells, leading to the suppression or enhancement of the tumor growth, respectively. Herein, we aimed to evaluate the interaction between breast cancer cell lines and GrB-producing B cells isolated from breast cancer-draining lymph nodes.

Methods: We isolated mononuclear cells from fresh axillary lymph node samples using Ficoll-Hypaque gradient centrifugation. Lymphocytes were co-cultured with breast tumor cell lines (MCF-7 and MDA-231) in the presence of recombinant IL-21 and Anti-BCR and then we measured the production of granzyme B in B cells and the apoptosis of tumor cells (MCF-7) by flow cytometry and Calcein release assay, respectively.

Results: Our analysis showed that direct co-culture of lymphocytes with either MDA-231 or MCF-7 cancer cells resulted in a significant reduction in the frequency of GrB-producing B cells. However, 24 hours stimulation of B cells with IL-21 and anti-B cell Receptor (BCR) prior to co-culture rescued GrB production. The supernatant of the two cell lines did not significantly change the B cells' expression of GrB. Moreover, B cells activated with IL-21 and anti-BCR induced significant apoptosis in MCF-7 cells.

Conclusion: These experiments showed that GrB-producing B cells and tumor cells have a bidirectional relationship that can be manipulated to enhance antitumor immunity.

Keywords: Granzyme B, B cells, Tumor-draining lymph node, Breast cancer

IL-38 Serum Levels in Iranian Women with Breast Cancer: A Case-Control Study

Aida Khademolhosseini[1], Mohammad Reza Haghshenas[1], Seyedeh Niloufar Mousavi[1], Soolmaz Khansalar[1], Abbas Ghaderi[2]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran; Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

Background: Breast cancer (BC) is the world's most common cancer in women worldwide. Although being over 40 years old is a significant risk factor for BC, the average age of BC occurrence in Iran is approximately 10 years lower than in developed countries. Interleukin-38 (IL-38) is a novel member of the IL-1 family of cytokines with anti-inflammatory properties. However, the role of IL-38 in the pathogenesis of breast cancer has not yet been investigated. This study aimed to investigate IL-38 serum levels and their associations with the clinicopathological characteristics in patients with breast cancer in two age groups $\leq$ 40 and $\geq$ 50 years old.

Methods: In this case-control study, 60 patients with breast cancer were categorized into two age groups $\leq$ 40 and $\geq$ 50 years old, each containing 30 patients. Additionally, thirty healthy individuals were enrolled as a control group. IL-38 serum levels were measured by enzyme-linked immunosorbent assay (ELISA) in patients and controls.

Results: Our results indicated that IL-38 serum levels were significantly lower in patients than in healthy controls ($p=0.007$). Serum levels of IL-38 in patients with ages $\leq$ 40 were significantly lower than in healthy individuals in the same age group ($p=0.003$). No significant associations were found between IL-38 serum levels and the clinicopathological characteristics of the patients.

Conclusion: The present study revealed that IL-38 serum levels were lower in patients with BC, especially in patients with $\leq$ 40 years of age. A decrease in IL-38 serum concentrations might have effects on anti-inflammatory mechanisms in BC. However, our findings need to be confirmed by further studies with larger sample sizes.

Keywords: Breast cancer, IL-38, Age

Immune checkpoint and epithelial-mesenchymal transition gene expressions in esophageal squamous cell carcinoma; A computational study

Mehdi Montazer[1], Majedeh Ramian Yarasmi[2], Mehran Gholamin[3]

1. Department of Pathology, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Department of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran.
3. Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran

Background: The present computational study aimed to verify the findings of our previous clinical investigation on the immune checkpoint (IC) and epithelial-mesenchymal transition (EMT) gene expressions.

Methods: Clinicopathologic and RNA-Seq gene expression data of esophageal squamous cell carcinoma (ESCC) patients were extracted from The Cancer Genome Atlas (TCGA) database. The desired genes were CTLA4, PD1, PDL1, TIM3, and LAG3 (IC genes), and TWIST1 and MMP13 (EMT genes). Kaplan-Meier (KM) method, COX Proportional Hazard, and KM Plotter (a web-based tool) were employed for survival analysis and generating the prognostic model. Analyses were conducted in RStudio 2.22.0.7.0. Functional protein networks were investigated by STRING in Cytoscape software.

Results: Clustering perfectly separated two clusters, ICs and EMTs, based on pairwise correlations. PDL1 formed a distinct subcluster within the IC cluster, and interestingly, TIM3 was the only IC gene presenting a significant correlation with both EMT gene expressions (r=0.3, *p-value<0.01*). Gene expressions were statistically unrelated to clinicopathologic variables. Higher expressions of PDL1 (HR, 95%CI, p-value: 4.17, 1.81-9.64, 0.001), and MMP13 (3.43, 1.28-9.19, 0.014), lower expression of TWIST1 (0.22, 0.08-0.67, 0.008), and an advanced stage (2.26, 1.04-4.89, 0.039) were independently associated with a poor prognosis. Although the KM plotter revealed a merely marginal prognostic effect for TWIST1 in most cancers (HR<2), it was linked to better survival in some cancers, including esophageal and lung SCC. TWIST1, MMP13, and IC genes belonged to three distinguished protein networks.

Conclusion: We propose TIM3, which is generally categorized as an IC gene, to play a role in EMT. Besides PDL1, with well-recognized clinical consequences and targeted treatment, MMP-13 and TWIST1 should also be considered as promising biomarkers in ESCC. Moreover, we believe that TWIST1 is probably a double-edged sword leading to contradictory prognostic behaviors. More evaluation of TWIST1's role is thus required, especially on tumors with squamous differentiation.

Keywords: esophageal squamous cell carcinoma, immune checkpoint, epithelial-mesenchymal transition, prognosis

Immunoproteomics Analysis for Identification of Novel Biomarker as a tumor-associated antigen in Bladder Cancer Patients

Samira Tabaei[1], Mohammadreza Haghshenas[2], Kambiz Gilani[3], Allan Stensballe[4], Abbas Ghaderi[5]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Integrative Oncology Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
4. Department of Health Science and Technology, Aalborg University, 9200, Aalborg Ø, Denmark
5. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Exploration of tumor-associated antigen from bladder tumor cell line and identification of candidate autoantibody as clinical biomarkers for early detection of bladder cancer (BC).

Methods: Proteins isolated from JAM-ICR cells were subjected to immunoproteomics using pooled serum samples of patients diagnosed in different stages of BC (n= 35) and healthy control (n=30). Immunoreactive proteins were identified by liquid chromatography-mass spectrometry analysis. In silico analyses were performed to characterize any association of these proteins with BC.

Results: Seven protein tumor antigens that were reactive with serum patients of UBC were detected. The Immune reactivity pattern of the JAM-ICR cell line with BC patients' sera was different in various stages. Among these, 2 proteins reacted with serums in early and advanced BC stages, 3 of them reacted with early-stage serums, and 2 proteins just reacted with serum in patients under 50 years old. Interestingly, none of the 35 healthy control serums demonstrated tumor-associated antigens.

Conclusion: We identified seven autoantibodies as novel biomarkers for early diagnosis and diagnostic accuracy between the early and advanced stages of BC. Also, measuring these autoantibodies in a patient's serum may represent a simple and very useful approach for fast-tracking detection and targeted therapy of patients with highly aggressive BC.

Keywords: Urothelial bladder cancer, Cell line, Immunoproteomics, Biomarker

Importance of Nectin2, NUF2, and Nectin4 Genes Expression in Pathogenesis of Different Subtypes of Breast Cancer

Zahra Roshanizadeh[1], Abbas Ghaderi[1], Mohammad Reza Haghshenas[1], Amin Ramezani[2]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Targeted therapy by breast cancer-associated biomarkers has minimized the side effects of breast cancer treatment procedures. It has been reported that noble alternative immune checkpoints (e.g., Nectin2 and Nectin4), as well as cell division markers (e.g., NUF2), may affect the pathogenic features and the outcome of the therapeutic processes in cancer.

Methods: We investigated the expression of Nectin2, Nectin4, and NUF2 genes by Real-time PCR assay in 46 tumor tissues obtained from breast cancer and 46 adjacent tissues as the control group.

Results: Our results indicated a significant increase in the expression of the NUF2 gene in tumoral tissues in comparison to adjacent normal tissues (p=0.005, fold change=3.7). Although, no statistically significant difference was found between tumoral and adjacent tissues for the Nectin2 and Nectin4 genes. However, the expression of Nectin2 was significantly associated with lymph vascular and perineural invasion. The results also showed the higher expression of Nectin2 in the early stage of the disease as well as subtypes with estrogen receptor+ (ER+), progesterone receptor+ (PR+), and human epidermal growth factor-(HER2-). Additionally, the expression of NUF2 and Nectin4 was higher in advanced-stage and triple-negative breast cancer (TNBC) subtypes. Finally, the expression of these three genes was higher in patients aged<=45 years.

Conclusion: Our results show that levels of NUF2, Nectin2, and Nectin4 gene expression may influence the initiation, progression, and pathogenesis of breast cancer subtypes.

Keywords: Biomarker, NUF2, Nectin2, Nectin4

In vitro and In vivo Anticancer Activity of Mebendazole on Colon Cancer

Amin aliabadi[1], Nasrullah Erfani[2], Mohamad Reza Panjeshahin[1], Mohammad Reza Haghshenas[1], Razie Kiani[1]

1. Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Iran

Background: Colorectal cancer (CRC) is the third most common cancer and one of the leading types of cancer that result in death worldwide. Therefore, new treatment methods with better efficiency and fewer risks are essential. Mebendazole (MBZ), a drug commonly used for helminthic infections, has recently received attention as a suitable candidate for the treatment of various cancers. This study aimed to investigate, in vitro and in vivo, the anticancer activity of MBZ on colon cancer.

Methods: HT-29 (Human Colorectal Adenocarcinoma) and MCF-10 (non-tumorigenic epithelial) cell lines were treated with Mebendazole and Doxorubicin (Positive control drug). IC50 values were estimated using methyl thiazole diphenyl-tetrazolium bromide (MTT) assay. Flow cytometry was employed to evaluate the mechanism of cell death at IC50 values using Annexin V, FITC Apoptosis Detection Kit. For the animal study, colon cancer was induced by CT26 cells (mouse colon cancer) subcutaneously in BALB/c mice. Ten male mice were treated with MBZ as (0.05 LD 50, IP, Every other, 35 Days). In the end, the tumor size and weight were calculated.

Results: Data from three independent MTT experiments in triplicate revealed that IC50 values after 72 hours for HT29 and MCF-10 cell lines were 0.29±0.04 µM and 0.80 ±0.02 µM, respectively. Annexin V/PI staining demonstrated that Mebendazole treatment at IC50 concentrations induced 78±12 % ($p \leq 0.0001$) apoptosis in the HT29 cancer cell line after 48 hours, while the percentage of apoptosis in MCF-10 normal cells was 4±05 %. Also, in the animal study, mebendazole significantly reduced tumor size (1177 ±1109 mm3; $p \leq 0.001$) and weight (2.30±1.97gr; $p \leq 0.0001$) compared to the negative control group (weight 12.45±2gr; volum7346±1077 mm3).

Conclusions: Mebendazole strongly and selectively inhibits proliferation and induces apoptosis in colon cancer cells. Therefore, it might be a promising drug for clinical investigations.

Keywords: Mebendazole, Cancer, Colon cancer

In vitro and in vivo evaluation of the anti-tumoral effect of M1 phenotype induction in macrophages by miR-130 and miR-33 containing exosomes

Maryam Moradi Chaleshtori[1], Mojgan Bandehpour[2], Sara Soudi[3], Samira Mohammadi Yeganeh[4], Seyed Mahmoud Hashemi[5]

1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
4. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: In the tumor microenvironment, macrophages polarize into the M2 phenotype to facilitate tumorigenesis. Tumor-derived exosomes can act as mediators between the tumor microenvironment and stromal cells by transporting proteins, mRNAs, and miRNAs. Exosomal miRNAs play a pivotal role in modulating tumor microenvironment and macrophage polarization.

Methods: Here, we overexpressed miR-130 and miR-33 in exosomes of MDA-MB-231 cells and investigated their effect on macrophage polarization and tumor progression. For this purpose, exosomes were extracted from MDA-MB-231 cells and characterized using dynamic light scattering, electron microscopy, and western blotting of exosomal markers. Then, miR-130 or miR-33 containing exosomes were used to treat IL4-induced M2 or tumor-associated macrophages (TAMs). After treatment, the polarization status of macrophages, including the expression of M1-specific genes, and the secretion of cytokines were evaluated. Finally, the conditioned medium from exosome-treated macrophages was incubated with cancer cells to evaluate its effect on the migration and invasion ability of cancer cells and, in vivo experiments, investigated the effect of exosome-treated macrophages on breast cancer progression.

Results: Exosome characterization results approved the range of size and homogeneity of extracted exosomes. Overexpression of miR-130 and miR-33 in exosomes increased the expression of M1 signature genes (IRF5, MCP1, CD80) and secretion of cytokines (IL-1β and TNF-α) as well as yeast phagocytic activity of macrophages. Besides, the conditioned medium of macrophages treated with miRNA-containing exosomes declined the migration and invasion ability of cancer cells. The in vivo results indicated the inhibitory effect of exosome-treated macrophages on tumor growth. Furthermore, the results showed that in response to exosome-treated macrophages, the production of TNF-α by spleen cells increased, while the production of IL-10 and TGF-β by these cells decreased.

Conclusion: These findings suggest that overexpression of miR-130 and miR-33 in exosomes can decrease tumor progression by shifting macrophage polarization from M2 to M1 phenotype and can be a potential therapeutic strategy for tumor interventions.

Keywords: Breast cancer, Exosome, Macrophage polarization, miRNA

Inhibiting cancer cells with Echinococcus granulosus antigen B (EgAgB): A systematic review

Soheil Sadr[1], Hadi Mohebalian[2], Abbas Rahdar[3], Nooshinmehr Soleymani[2], Hassan Borji[2]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Physics, University of Zabol, Zabol

Background: Echinococcus granulosus is a parasitic tapeworm that causes echinococcosis or hydatid disease in humans and livestock. The larval stage of the parasite results in the formation of hydatid cysts in various organs, leading to serious health complications. A protein called Echinococcus granulosus antigen B (EgAgB) is present in the fluid of these hydatid cysts. This review aims to examine prior literature on tumors and Echinococcus granulosus.

Methods: Nine databases were searched for published articles on Echinococcus granulosus anti-tumor effects from January 2000 to January 2023. Fifteen relevant articles with complete abstracts were included in the study. R version 4.2.1 artificial intelligence software was utilized to extract and analyze data from interconnected papers.

Results: The findings indicate that EgAgB exhibits anti-tumor effects in various types of cancer, including colon, lung, and breast cancer. One mechanism by which EgAgB exerts its anti-tumor effects is inducing programmed cell death or apoptosis in cancer cells, leading to the death of cancer cells and slowing or halting tumor growth. EgAgB has also been demonstrated to inhibit cell proliferation in cancer cells, which can impede the growth of tumors. Additionally, EgAgB stimulates the immune system to attack cancer cells, enhancing the protein's anti-tumor effects. EgAgB has been used in combination with other anti-cancer agents to enhance their efficacy. For example, in one study, the combination of EgAgB and doxorubicin (a chemotherapy drug) resulted in a synergistic effect in inhibiting the growth of breast cancer cells.

Conclusion: While the anti-tumor effects of EgAgB are encouraging, additional research is necessary to comprehensively comprehend its potential as a cancer treatment and optimize its clinical use. Overall, the findings suggest that EgAgB may be a promising candidate for cancer treatment and warrant further investigation.

Keywords: Antitumor, Apoptosis, Cancer immunotherapy, Echinococcus granulosus

Inhibition of Cyclooxygenase-2 enhances anti-cancer responses in breast cancer: modulation of Cancer-Associated Fibroblasts

Arash Samoudi[1], Firoozeh Abolhasani-Zadeh[2], Ali Afgar[3], Elnaz Jalilian[1], Ladan Langroudi[1]

1. Department of Medical Immunology, School of Medicine, Kerman University of medical sciences, Kerman, Iran
2. Department of Surgery, School of Medicine, Kerman University of medical sciences, Kerman, Iran
3. Department of Parasitology and Mycology, School of Medicine, Kerman University of medical sciences, Kerman, Iran

Background: Cancer-Associated Fibroblasts (CAFs) as the main constituent of the tumor microenvironment play an important role in the progression of the tumors. Production of PGE2 is one of the mechanisms by which these cells contribute to the suppression of the immune system. Therefore, in this study, by using celecoxib, we evaluated the inhibition of the COX-2 enzyme in CAFs to study the profile of the PBMCs in co-culture with the CAFs treated with Celecoxib.

Methods: Blood samples and Tumor tissue of invasive ductal carcinoma of the breast in stages 2 and 3 were collected. CAFs were isolated using the explant method and verified using the expression of FAP-1 with ICC. CAFs were treated with Celecoxib and cultured with the patient's PBMC. MTT test was used to evaluate Lymphocyte proliferation. The level of IL-4, PGE2, TGF-β, IL-10 and IFN-γ was evaluated using Elisa. To assess the expression of T-bet, GATA3, and FOXP3 transcription factors as well as COX-2 and α-SMA, a real-time PCR test was carried out.

Results: The secretion of PGE2 and the expression of the α-SMA, one of the important markers of CAFs was significantly reduced (p=0.002 and p=0.007 respectively). Among the transcription factors, the expression of T-bet significantly increased (p=0.02) and also expression of FOXP3 was significantly decreased (p=0.05). Among the cytokines, the secretion of IL-10 (p=0.001) and TGF-β (p=0.02) in lymphocytes was significantly decreased, and the level of IFN-γ (p=0.002) was significantly increased.

Conclusion: The expression of COX-2 in many cancers has been recognized as an important mechanism in cancer progression and immune suppression. CAFs are one the main producers of COX-2 in the TME. Inhibition of COX-2 in CAFs was able to reverse the CAF phenotype and enhance the anti-tumor profile in PBMCs. Thus, targeting of CAFs, as supporters of cancer progression, could show beneficial in combined immunotherapy of cancers.

Keywords: CAF, PGE2, COX-2, Celecoxib, IFN-γ

Inhibition of hypoxia-induced metastasis using an HRE-harboring vector

Maryam Sadri[1], Ali Akbar Delbandi[1], Nesa Rashidi[1], Mohammad Reza Bolouri[1], Reza Falak[1], Ghollam Ali Kardar[2]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

Background: Hypoxia is the main characteristic of most solid tumors. The hypoxia-inducible transcription factor (HIF) is a well-known and effective hypoxia regulatory factor and its expression can affect tumor biology. HIF binds to the Hypoxia response element (HRE) sequences in the promoter region of various genes and through inducing their expression, increases tumor survival, invasion, angiogenesis, and epithelial-mesenchymal transition (EMT). In this study, we designed a plasmid construct consisting of a HIF-binding sequence, capable to bind the proximal promoter of the Snail gene, and its inhibitory effect on cancer metastasis under hypoxic conditions was investigated.

Methods: Cobalt chloride (CoCl2) was used to induce hypoxia in the studied cells. SKBR3 cells were transiently transfected with pIRES2-EGFP (control) or Snial-HRE-pIRES2 (construct) vector and treated with 150 micM CoCl2 for 48 h. The effect of the vectors on the inhibition of the HIF-1 downstream genes was evaluated using qRT-PCR and western blotting.

Results: Hypoxia induction increased the expression of VEGFA and HIF-1α in SKBR3 cells; also, upregulated the expression of N-cadherin, β-catenin, Snail, and vimentin and downregulated the expression of E-cadherin in those cells, indicating EMT occurrence. The increased MMP2 and MMP9 expression of the construction-transfected SKBR3 cell revealed their increased invasion capacity at the hypoxic condition. Moreover, they showed a decrease in the expression of Snail, N-cadherin, VEGF-A, vimentin, and β-catenin, demonstrating the inhibition of angiogenesis and EMT. Also, a decrease in the expression level of invasion-related genes, including MMP2 and MMP9, was observed.

Conclusion: During hypoxia, the produced HIF could bind to the HRE sequence in the designed construct or present in the host cell gene promotors. We found that this competition prevented the binding of the HIF factor to the promoters of other genes and hypoxia-induced mechanisms such as EMT, angiogenesis, and invasion were inhibited. Regulation of hypoxia-inducible gene expression using DNA-binding sequences may represent a new approach for targeting hypoxia-induced metastasis in the future.

Keywords: Cobalt chloride, Hypoxia, Hypoxia-inducible factor, Hypoxia response element

Intra-lesion injection of activated NK cells in recurrent malignant brain tumors

Marzieh Ebrahimi[1], Niloufar Shayan Asl[1], Maryam Behfar[2], Rouzbeh Shams Amiri[3], Rashin Mohseni[2], Masoumeh Azimi[6], Yalda Jafari[1], Mohammad Faranoush[4], Amirhossein Izadpanah[1], Monireh Mohmmad[1], Amir Ali Hamidieh[5], Zohreh Habibi[6]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Pediatric Cell and Gene Therapy Research Center, Gene, Cell and Tissue Research Institute, Tehran University of Medical Sciences, Tehran, Iran
3. Neuroscience Research Center, Golestan University of Medical Sciences, Gorgan, Iran
4. Pediatric Growth and Development Research Center, Iran University of Medical Sciences, Tehran, Iran
5. Children's Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran
6. Department of Pediatric Neurosurgery, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran

Background: Despite multi-modal therapies for patients with malignant brain tumors, their median survival is <2 years. Recently, NK cells provided cancer immune surveillance through their direct natural cytotoxicity by modulating dendritic cells to enhance the presentation of tumor antigens and regulating T-cell mediated antitumor responses. However, the success of this treatment modality in brain tumors is unclear. Our previous pre-clinical study showed that intracranial injection of activated haploidentical NK cells is safe and resulted in the eradication of glioblastoma tumor masses in the rat model. Therefore, in the present study, we evaluated the safety of intra-surgical cavity or intra cerebrospinal fluid (CSF) Injection of ex vivo activated haploidentical NK cells in patients with recurrent glioblastoma multiform (GBM) and malignant brain tumors resistance to chemo/radiotherapy.

Methods: This study is a single-arm, open-label, investigator-initiated phase I clinical trial in recurrent malignant brain tumors, including GBM. The study included six patients. Depending on tumor size, 2x106 up to 100x106 pre-activated haploidentical CD56+ cells that were injected directly into the tumor bedside using implanted reservoirs or intra CSF, 3 times. The patients were followed up by the neurosurgeon who performed the injection and an independent clinical assessor.

Results: Any adverse reactions were not observed post each injection. Among 6 patients, one case had a complete response, two cases had stable disease for three months post-last injection, and three of them had progressive disease.

Conclusion: The results showed that local administration of the haploidentical NK cells in malignant brain tumors is safe, feasible, tolerated at a higher dose, and also is cost-effective. Trial registration: IRCT, IRCT20170122032121N5. Registered 2020-06-11- prospectively registered, https://www.irct.ir/trial/47366

Keywords: Brain tumor, Glioblastoma multiform, Haploidentical, Malignant, NK cells.

Investigating the expression of immune checkpoints in tumor tissues and adjacent normal tissues in the women referred to Imam Reza Hospital in Tabriz in 2019

Hamid Mavaddati[1], Behzad Baradaran[2], Mostafa Haji Molahoseini[3]

1. Tabriz Immunology Research Center, Tabriz. Iran.
2. Tabriz University of Medical Sciences and Medical Services, Tabriz. Iran.
3. Shahid Beheshti University of Medical Sciences and Medical Services, Tehran, Iran.

Background: Treatments with immune checkpoint blockade bring notable clinical benefits to fighting several solid cancers. Nevertheless, the effectiveness of immune checkpoint blockade in breast cancer remains controversial. Immunotherapies targeting PD-1 and CTLA-4 have elicited promising responses in several cancers. However, the relatively low response rates warrant the investigation of additional immunosuppressive pathways. High expression of immune checkpoints in the tumor microenvironment plays a significant role in inhibiting anti-tumor immunity, which is associated with poor prognosis and cancer progression.

Methods: In this study, we collected tumor tissue samples and adjacent-normal tissue samples from 50 people with breast cancer. Then we evaluated the expression level of immune checkpoints including VISTA, TIGIT, B7H3, B7H6, and B7-H7 (HHLA-2) in these tissues.

Results: Our results revealed that expression levels of VISTA, TIGIT, B7H3, B7H6, and B7-H7 (HHLA-2) were significantly altered in breast tumor tissues, in comparison with breast adjacent-normal tissues. We also found that expression of all the abovementioned genes was significantly altered in triple-negative breast cancer compared to normal tissue.

Conclusion: Collectively, our data revealed that these genes may be a diagnostic or therapeutic biomarker in breast cancer therapy.

Keywords: Breast cancer therapy, Immune checkpoint expression, Triple-negative breast cancer

Investigation of Pro-Inflammatory Cytokines (TNF-α, IL1-β) Levels and Total Reactive Oxygen Species (ROS) in Human Hepatoma Cell Line (HepG2) Treated with Methanolic Moringa Oleifera Leaves Extract

Asieh Emami Nejad[1]

1. Payame Noor University, Iran

Background: In recent decades, the lack of control of cancer by chemical drugs and its side effects have made this disease become one of the biggest problems in the world. Medicinal plants are considered the main factors of complementary medicine. Many plant compounds have high anti-cancer. Moringa oleifera (MO) is one of the medicinal plants with numerous medicinal properties such as anti-cancer and anti-inflammatory. The current study was conducted to investigate the cytotoxicity, anti-inflammatory and antioxidant effects of the methanolic extract of Moringa oleifera leaves on HepG2 human liver cancer.

Methods: First, quercetin as the main compound of the methanolic extract of Moringa oleifera leaf was purified by high-performance liquid chromatography (HPLC). HepG-2 cell line were treated with concentrations (0, 30, 40, 50, 60, and 70 µg/mL) of methanol extract of Moringa oleifera leaves, pure quercetin (50 µg/mL), and doxorubicin (1 µg/mL). After 48 hours, the cytotoxic activity of different concentrations of methanolic extract of Moringa oleifera leaves on the HepG2 cell line was evaluated by the MTT method. Also, total cellular ROS were determined using in adherent cells using 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and Pro-inflammatory cytokines such as TNF-α and IL-β measurement by (ELISA).

Results: In this study, IC50 values of Methanolic leaf extract were determinant (IC50 = 12.89 µg/mL) on HepG2 cell lines. Survey of cytotoxicity effects of Moringa oleifera demonstration that HepG2 viability was concentration-dependent. Comparison of the effect of the MO extract with doxorubicin and pure quercetin (as positive control) showed that the effect of the concentration of 70 µg/mL methanolic extract and pure quercetin and doxorubicin on liver cancer cells was similar ($p>0.05$). Also, the results showed that methanolic leaf extract has cytotoxic effects on the HepG-2 cell line and inhibits the growth of liver cancer cells. Also, methanolic Moringa oleifera decreased the production of pro-inflammatory cytokines (TNF-α, IL-1β) versus untreated HepG-2 cell culture ($p<0.001$). Total ROS production showed an increase in HepG-2 cells treated with Methanolic Moringa oleifera in comparison to untreated HepG-2 cells ($p<0.001$).

Conclusion: Finally, the results of this study demonstrated Methanolic Moringa oleifera extracts exerted inhibitory effects against the HepG-2 cancer cell line, also, the anti-inflammatory and antioxidant effects of this extract can introduce it as one of the effective substances in cancer control. Although more research is needed to clarify the effects of the Methanolic Moringa oleifera leaf extract.

Keywords: Cytotoxicity, Moringa oleifera, Quercetin, Human Hepatoma Cell (HepG-2), MTT Assay, Pro-inflammatory cytokines (TNF-α, IL-1β), ROS, ELISA

Investigation of SMAD4 expressions in patients with colorectal cancer and in the adjacent control group

Reyhaneh Yarmohammadi[1], Zahra Mozooni[2]

1. Doctoral Student Carolina University Winston-Salem, NC, USA
2. Institute of Immunology and Infectious Diseases, Antimicrobial Resistance Research Center, Iran University of Medical Sciences, Tehran, Iran

Background: Colorectal cancer (CRC) is the third deadliest cancer worldwide. SMAD4 is the common mediator of important transforming growth factor-β (TGFβ) signaling and has an impact on colorectal cancer. Moreover, SMAD4 protein expression approximately decreases in colorectal cancer and is associated with poor response to chemotherapy and metastasis formation. In addition, SMAD4 plays an important role in cell functions such as apoptosis and cell proliferation. Moreover, SMAD4 has inactivating mutations in some cancers, and decreased expression is a major feature of most human cancers. As well as previous studies have found a correlation between SMAD4 gene expression and patient survival and disease progression of CRC. In the present study, we aimed to investigate the expression of SMAD4 in CRC and adjacent control tissues.

Methods: Quantitative Real-Time PCR (qRT-PCR) method was used to investigate the expression of SMAD4 in 90 colorectal tumor tissues and adjacent control tissues. In addition, we analyzed the diagnostic significance of the aforementioned SMAD4 by plotting the receiver operating characteristic (ROC) curve.

Results: Our results revealed that the expression level of SMAD4 was significantly down-regulated in CRC patients compared with the adjacent control group ($p<0.0001$).

Conclusion: These results suggest that SMAD4 levels may be useful as a potential diagnostic biomarker for CRC.

Keywords: Colorectal cancer (CRC), SMAD4, Diagnostic biomarkers, Real-Time PCR (qRT-PCR)

Investigation of soluble and full-length CTLA-4 mRNA in peripheral blood of patients with breast cancer

Razie Kiani[1], Aida Khademolhosseini[1], Sedigheh Tahmasebi[2], Zahra Faghih[1], Nasrollah Erfani[3]

1. Cancer Immunology group, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Cancer Immunology group, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran; Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: CTLA-4 is a negative regulator of the immune system. As an immunotherapy approach, blocking CTLA-4 improves anti-tumor immunity. Besides full-length (fl) CTLA4 which is expressed on the cell surface, the soluble form of CTLA4 (sCTLA4) attracted attention in recent years. This study aimed to investigate the expression levels of sCTLA4 and flCTLA4 mRNA in the peripheral blood of patients with breast cancer and to understand how these levels are related to breast cancer progression and response to treatment.

Methods: A total of 50 patients with breast cancer without prior treatment and 30 healthy controls were recruited RNA was extracted from peripheral blood and cDNA was synthesized using reverse transcription. The mRNA expression levels of sCTLA4 and flCTLA4 were measured using quantitative real-time PCR (RT-PCR) and normalized to the expression level of the reference gene GAPDH. Furthermore, the association of sCTLA4 and flCTLA4 expression levels with clinical and pathological parameters, including tumor stage, grade, and lymph node metastasis was also evaluated.

Results: The results showed that the expression levels of sCTLA4 were significantly higher in patients with breast cancer compared to healthy controls (mean ± SD: 0.76 ± 0.23 vs. 0.50 ± 0.15, $p<0.001$), while the expression levels of flCTLA4 did not differ significantly between the two groups (0.93 ± 0.35 vs. 0.96 ± 0.27, $p=0.69$). Further analysis showed that the expression levels of sCTLA4 were significantly higher in patients with advanced-stage breast cancer (stages III and IV) compared to early-stage breast cancer (stages I and II) (0.85 ± 0.20 vs. 0.71 ± 0.24, respectively; $p=0.03$). Moreover, the expression levels of sCTLA4 were significantly higher in breast cancer patients with lymph node involvement (2.46 ± 0.62) compared to patients without lymph node involvement (1.76 ± 0.54) ($p=0.023$). In contrast, there was no significant difference in the expression levels of flCTLA4 between the two groups ($p=0.287$).

Conclusion: These findings suggest that sCTLA4 may play a role in the development and progression of breast cancer with lymph node involvement. Additionally, sCTLA4 may be a potential biomarker for breast cancer, particularly in advanced stages. Further studies are needed to investigate the functional role of sCTLA4 in breast cancer and to evaluate its potential as a therapeutic target.

Keywords: Breast cancer, sCTLA4, fCTLA4, Biomarker

Investigation of the Frequency of SNPs (rs 1049174 G>C (of the NKG2D Receptor on Natural Killer Cells in Various Types of Breast Cancers and Correlation with IL-6, IL-8, TNF-α Levels

Behzad Karami[1]

Background: Breast cancer is the most common women's cancer worldwide. Natural killer (NK) cells are natural cytotoxic cells, effector cells of the innate immune system. These cells play an essential role in the defense against cancer cells. NKG2D receptors are one of the vital receptors in NK cells. In humans, the NKG2D gene is two single nucleotide polymorphism SNPs (rs 1049174 G>C). GG SNP makes a high-affinity receptor for NKG2D ligands whereas, CC polymorphism reduces NKG2D affinity for their ligands. The aim of this study is a survey of the frequency of SNPs (rs 1049174 G>C (of the NKG2D in Ductal carcinoma, estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) breast cancer.

Methods: In this Case- Control research, 100 breast cancer patients (36 Ductal Carcinoma,22 estrogen receptors (ER), 18 progesterone receptors (PR), and 24 human epidermal growth factor receptor 2 (HER2) and 100 healthy women were selected from breast cancer care center (Iran, Yazd). RFLP-PCR assay using specific primers for NKG2D gene polymorphism (SNP) (rs 1049174 G>C) was performed Also serum pro-inflammatory cytokines (IL-6, IL-8, TNF-α) measurement by ELISA kit. Statistical analysis was done using SPSS version 22, and GraphPad Prism version 8 software.

Result: The result of this research demonstrated that the frequency of CC and GG, GC genotypes in the patients were 71.3%, 22.2%, and 6.5% versus 44.4%, 42.6%, 13% respectively in comparison to healthy women (p<0.05). CC genotype has more susceptibility to breast cancer in the comparison control group (CI=95%, 1.31-2.24, OD=1.6). Also, 71% of Ductal carcinoma, 45% ER+,34% PR+, and 68% HER-2+ breast cancer patients have CC genotype (SNP) (rs 1049174 G>C). On the other hand, the amounts of pro-inflammatory Cytokines IL-6 (26.64±13.12 pg/ml), IL-8 (123.05±11.02 pg/ml), TNF-α (48.68±21.93 pg/ml) in the breast cancer CC genotype have increased significantly statically compared to the other genotype (GG, GC) and the control groups (p<0.05).

Conclusion: Results showed that homozygote CC genotype SNP (rs 1049174 G>C) can be increased the risk of breast cancer malignancy and correlate with severe patients' situations. Since SNP (rs 1049174 G>C) genotype is on the NKG2D receptor of the NK cells, which is the vital barrier for immune response to defense against the cancer cells, women with the CC genotype have a low affinity for binding to the ligand (MICA/MICB), resulting in increased inflammation through the production of pro-inflammatory cytokines (IL-6, IL-8, TNF-α).

Keywords: Breast cancer, Natural Killer Cells, NKG2D, SNP, pro-inflammatory Cytokines (IL-6, IL-8, TNF-α)

Investigation of the Protein Expression and Prognostic Significance of PD-1, PD-L1, CD45RO, and MMR in Gastric Cancer Tissues

Niloufar Namvar[1], Abbas Ghaderi[1]

1. Shiraz University of Medical Sciences, Shiraz, Iran

Background: There is an urgent need to discover novel prognostic biomarkers and treatment strategies for gastric cancer (GC) patients. Several immune-related markers have been proposed as prognostic tools and immunotherapeutic targets to manage diseases. In this regard, we evaluated the expression pattern and prognostic significance of programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1), CD45RO+ tumor-infiltrating lymphocytes (TILs), and DNA mismatch repair (MMR) proteins (MLH1, MSH2, PMS2, and MSH6) in non-metastatic intestinal-type gastric adenocarcinoma (GAC).

Method: Samples and data from 70 GC patients were retrospectively collected. Immunohistochemistry staining was used for the detection of the markers mentioned above. We then evaluated the prognosis significance of each marker and their intercorrelation.

Results: Cytoplasmic PD-1 was expressed by tumor cells (TCs) and was significantly associated with poorer survival (p= 0.012). However, multivariate analysis indicated that TNM-stage, tumor location, and extracellular mucin showed stronger prognostic values (p<0.001 and 0.003 and 0.011, respectively). A significant positive association was found between CD45ROhi TILs and PD-1 expression on tumor-infiltrating cells (TICs) (p= 0.026). All GC patients with deficient MMR (d-MMR) had a higher number of CD45RO+ TILs and were associated with PD-1+ TICs and PD-L1+ TCs, although the difference was not statistically significant.

Conclusion: Despite the significant association of PD-1 overexpression on TCs with shorter overall survival (OS), histopathologic factors including tumor location, TNM stage, and extracellular mucin seem to be still the strongest prognostic factors in non-metastatic intestinal-type GAC. Additionally, our data support a prognostic impact for d-MMR and CD45RO but not PD-1 and PD-L1 expression on TICs.

Keywords: PD-L1, PD-1, DNA mismatch repair system, CD45RO, Gastric cancer, Immunohistochemistry

Investigation of the effect of ibuprofen on drug resistance of 5-fluorouracil (5-FU) in gastric cancer cell line

Mahdi Bagheri[1], Seyed Javad Hoseini[1], Hassan Akrami[2], Nasrollah Erfani[3], Amirhossein Ahmadi[4]

1. Department of Biological Science, Faculty of Nano and Biological Sciences and Technology, Persian Gulf University, Bushehr, Iran
2. Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Biological Science, Faculty of Nano and Biological Sciences and Technology, Persian Gulf University, Bushehr, Iran

Background: Combination therapies have presented significant achievements in preventing drug resistance in cancer. However, increasing drug resistance remains an important challenge in therapeutic drugs. In this study, we investigate the drug resistance of 5-fluorouracil (5-FU) in a gastric cancer cell line (AGS) treated with ibuprofen in the combination treatment model of two drugs.

Methods: The simultaneous effects of ibuprofen and 5-FU with different concentrations on AGS cell viability, cell proliferation, and apoptosis were investigated by MTT assay and DNA laddering assay for 24 and 48 h. The IC50 values of ibuprofen and 5-FU were calculated with GraphPad Prism software.

Results: The IC50 values for ibuprofen and 5-FU in AGS cells were 110 µg/ml and 0.8 µg/ml, respectively, and combination IC50 for ibuprofen and 5-FU were 85 µg/ml and 0.45 µg/ml, respectively. DNA laddering pattern was observed in AGS cells treated with 110 µg/ml and 0.8 µg/ml of ibuprofen, 5F-U, and a combination of them, respectively.

Conclusion: Altogether, our findings indicated that ibuprofen and 5-FU simultaneously reduce drug resistance in gastric cancer cell lines, by inducing apoptosis and inhibition of cell proliferation. The simultaneous use of these two drugs had a synergic effect and reduced drug resistance in cancer cells.

Keywords: Gastric cancer, Apoptosis, Drug resistance, 5-fluorouracil

Investigation of the role of cancer-associated fibroblasts in gastric cancer patients treated with nivolumab: a single cell RNA-seq study

Adib Miraki Feriz[1], Setareh Azarkar[1], Arezou Khosrojerdi[2], Hossein Safarpour[2]

1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran
2. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

Background: Gastric cancer (GC) is a worldwide health issue, with over one million new cases identified each year. Despite a five-decade drop in incidence and mortality, GC remains the third greatest reason for cancer-related death globally. Blocking immunological checkpoints, particularly programmed cell death-1 (PD-1) and its ligand (PD-L1 or B7-H1), has shown effectiveness in numerous solid tumors and appears to be a possible therapeutic option for GC. Cancer-associated fibroblasts (CAFs) protumor activities imply that they might be used as therapeutic targets in the treatment of cancer. The cause of CAF diversity in GC is unknown, which has hampered translational progress in CAF targeting. The purpose of this study was to investigate the effect of Nivolumab on CAFs subpopulations and their expression of inhibitory ICs, as well as the molecular connections between cells in the GC tumor microenvironment at single-cell RNA-seq resolution.

Methods: First, using the code GSE189926, raw single-cell sequencing data from patients who received nivolumab before (n=12) and after (n=10) therapy were retrieved from the NCBI database. For data processing, the Scanpy toolbox was used. Data from the quality control phase were first eliminated to remove dead cells, dividing cells, and stressed cells. Following that, the Scran package was used to conduct the Normalization step on the data. The batch effect was subsequently eliminated from the samples using the Combat package. Dimension reduction was subsequently performed using the PCA technique. Finally, cell clustering was conducted based on the expression of particular gene markers. The Bonferroni procedure was employed in statistical analyses to compute the Adjusted P-value.

Results: We found that there are two major sub-types of CAFs in GC TME based on gene expression profile including PDGFRA+ CAFs and PDGFRB+ CAFs. Also, our data revealed there is a high interaction between CAFs and CD8+ T cells via MIF/CD74 axis after nivolumab therapy. On the other hand, we examined the expression pattern of inhibitory immune checkpoints before and after nivolumab therapy.

Conclusion: Our data showed that the expression score of LAG3 and CTLA4 were increased after nivolumab therapy. Moreover, the cytotoxicity panel demonstrated the expression of all granzymes genes including GZMA, GZMB, GZMK, etc. were increased in post-treatment patients.

Keywords: Single-Cell RNA-seq, Nivolumab, Immune Checkpoints, Immunotherapy

Long non-coding RNA signatures and related signaling pathway in T-cell acute lymphoblastic leukemia

Homayoun Yousefi[1], Dariush Purrahman[1], Mohammad Jamshidi[2], Elena Lak[3], Bijan Keikhaei[4], Mohammad Reza Mahmoudian Sani[5], Hashem Kazemi[6]

1. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran
3. Department of Laboratory Sciences, School of Allied Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
4. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
6. Noor Medical Genetic Laboratory, Khuzestan Ahvaz, Iran

Background: T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy caused by clonal proliferation of T-cell pre-cursors arising from the thymus. Although the optimized chemotherapy regimen could improve the outcome of such patients, some challenges such as the higher risk for induction failure, early relapse, and isolated central nervous system (CNS) relapse occurring in T-ALL patients are of great significance, leading to increased mortality rates. Long non-coding RNA (lncRNA) is a key component involved in cell signaling through a variety of mechanisms in regulating gene expression.

Methods: Directories of open-access journals, Google Scholar, PubMed, EBSCO, and Web of Science were searched.

Results: Oncogenes and tumor suppressors are no exception and their expression can be affected by lncRNAs. In addition, accumulating research in samples from T-ALL patients as well as pre-clinical studies in mice suggest that the expression profile of lncRNAs in T-ALL could be aberrant, resulting in the deregulation of target genes and downstream signaling pathways. In addition, accumulating research in samples from T-ALL patients as well as pre-clinical studies in mice suggest that the expression profile of lncRNAs in T-ALL could be aberrant, resulting in the deregulation of target genes and downstream signaling pathways. These lncRNAs may be determinants of proliferation, apoptosis, and drug resistance observed in T-ALL.

Conclusion: lncRNAs can be a good tool to develop novel strategies against cancer cells in the treatment of relapsed and refractory T-ALL. They can also act as promoting biomarkers in assessing T-ALL and differentiating between patients with poor prognoses and good prognoses. Summary of the overall findings and the importance of the study Abstract Text Maximum 2000 Characters around 300 words.

Keywords: Drug resistance, long non-coding RNA, Prognostic, Signaling pathway, T-acute lymphoblastic leukemia.

MDSC depletion using a peptibody augmented inhibitory effects of an anti-HER2 monoclonal antibody on 4T1-HER2 tumor growth in vivo

Khadijeh Ramezani Ali-Akbari[1], Vahid Khaki-Bakhtiarvand[1], Jafar Mahmoudian[2], Hossein Asgarian-Omran[3], Fazel Shokri[4], Mohammad Hojjat-Farsangi[5], Mahmood Jeddi-Tehrani[6], Mahdi Shabani[1]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
4. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Oncology-Pathology, BioClinicum, Karolinska University Hospital Solna and Karolinska Institute, 17164 Stockholm, Sweden
6. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Background: Clinical efficacy of HER2 targeted strategies is limited due to impaired anti-tumor responses that are negatively affected by immunosuppressive cells. We thus, investigated the inhibitory effects of an anti-HER2 monoclonal antibody (1T0 mAb) in combination with MDSC depletion on the growth of 4T1-HER2 tumor in vivo.

Methods: BALB/c mice were challenged with a human HER2-expressing 4T1 murine breast cancer cell line. A week post tumor challenge, each mouse received 50 µg of an MDSC-specific antibody every other day, or 10 mg/kg of 1T0 mAb two times a week, and their combination for two weeks. The treatment's effect on tumor growth was measured by calculating tumor size. Also, the frequencies of MDSCs and T lymphocytes were measured by flow cytometry.

Results: Peptibody-treated mice indicated tumor regression and 40% of the mice eradicated their primary tumors. The peptibody was capable to deplete notably splenic MDSCs as well as intertumoral MDSCs ($p< 0.0001$) and led to an increased number of tumor-infiltrating CD8+ T cells (3.3 folds) and also that of resident tumor-draining lymph nodes (3 folds). The combination of peptibody and 1T0 mAb resulted in the enhanced expansion of tumor-infiltrating CD4+ and CD8+ T cells which were associated with tumor eradication in 60% of the mice.

Conclusion: Peptibody can deplete MDSCs in the 4T1-HER2 model and augments the anti-tumoral effects of the 1T0 mAb in tumor eradication. Thus, MDSCs have critical roles in the development and progression of tumors and their depletion is associated with the induction of anti-tumoral immune responses.

Keywords: 1T0 mAb, HER2, Cancer immunotherapy, MDSC, Peptibody, Tumor microenvironment

Micro RNA-34a increases carboplatin sensitivity in MCF-7 breast cancer cells by inducing apoptosis

Nazila Alizadeh[1], Behzad Baradaran[2]

1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Breast cancer (BC) has been classified among the main causes of death owing to female cancer. Carboplatin is a platinum-based chemotherapeutic drug that is an important treatment option for BC. But high and frequent doses of carboplatin usually reduce the reaction of cancer cells to medication. There is an immediate need to establish methods for increasing the carboplatin susceptibility to BC cells. For instance, micro RNAs (miRNAs) such as MiR34a demonstrate significant potential. Considering that, this research was planned to explore the better clinical effect and underlying mechanism of miR-34a as a possible tumor inhibitor and drug resistance regulator compared with carboplatin chemotherapy drug in the cell lines of BC in humans.

Methods: MCF-7 cell line was transfected with miR-34a to perform functional analyses. Subsequently, the MTT assay was applied to assess cell viability. Cell viability and cell death-associated gene expression amounts including Bax, Bcl-2, caspase-3, MDR1, P53, and mir34-a, were examined through quantitative real-time PCR.

Results: Findings showed that miR-34a upregulation significantly decreased MCF7 cell viability in comparison with the control group. Furthermore, the single treatment of cells with miR-34a mimics and carboplatin could significantly increase Bax, Caspase-3, and P53, and decrease in Bcl-2 mRNA expression levels compared to the non-treated group. Moreover, by reducing the expression levels of the MDR1 gene, BC cells' reaction to carboplatin has been increased via miR-34a.

Conclusion: This study showed, that miR-34a restoration might improve the responsiveness of breast cancer cells to carboplatin chemotherapy through the downregulation of MDR1.

Keywords: Breast cancer, miR-34a, Carboplatin, Apoptosis, Chemosensitivity.

miR-122 is more effective than rapamycin in the inhibition of Epithelial-to-Mesenchymal Transition and mTOR signaling pathway in triple-negative breast cancer

Majdedin Ghalavand[1], Samira Mohammadi-Yeganeh[2], Seyed Mahmoud-Hashemi[3]

1. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Medical Nanotechnology and Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The fundamental mechanism responsible for the aggressiveness of metastatic cancers such as triple-negative breast cancer (TNBC) is the Epithelial-to-Mesenchymal Transition (EMT). In cancer microenvironments, the PI3K-Akt-mTOR signaling pathway plays a critical role in regulating the EMT mechanism. Objective The current study focuses on the impacts of rapamycin, a newly repositioned chemotherapeutic agent against mTOR, and miR-122 on the aggressive behavior of TNBC.

Methods: The IC50 of rapamycin on 4T1 cells was determined using an MTT assay. Also, miR-122 was transiently transfected into 4T1 cells to study its effect on the pathway. Quantitative real-time PCR was conducted to assess the expression level of central mTOR and EMT-related cascade genes. Moreover, cell mobility and migration were evaluated using scratch and migration assays, respectively.

Results: Both rapamycin and miR-122 significantly decreased the expression level of PI3K, AKT, and mTOR, as well as ZeB1 and Snail genes. However, no significant change was observed in Twist's expression. Furthermore, scratch and migration assays revealed that the migration of 4T1 cells was markedly reduced, especially following miR-122 induction. Our experimental findings and gene enrichment studies indicated that miR-122 mainly operates on multiple metabolic pathways as well as EMT and mTOR, while rapamycin has restricted targets in the cancer cells.

Conclusion: Consequently, miR-122 can be considered a potential cancer microRNA therapy option, which can be validated in the future upon doing animal studies to demonstrate its efficacy in cancer control.

Keywords: Cancer microenvironment, miR-122, Rapamycin, RNA therapy, Signaling pathway

miR-34a loaded tumor-derived exosomes can effectively halt the cell cycle and invasion capacity of 4T1 cells.

Mahsa Hajivalili[1], Maryam Hosseini[2], Bahareh Niknam[3], Davar Amani[3]

1. Behbahan Faculty of Medical Sciences, Behbahan, Iran
2. Trauma Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Triple-negative breast cancer is the most invasive type of breast cancer with the highest rate of mortality. An important issue in this malignancy is its great potency for rapid proliferation. miR-34a has great oncosupressive effects in various cancer types. Tumor-derived extra-cellular vehicles could be considered as possible vehicles for miRNA- perfect delivery. In this research, we aimed to investigate the effects of miR-34a enriched tumor-derived exosomes (TEXs) on 4T1- cells.

Methods: 4T1 cells were cultured and the supernatant was collected after 48 hours (hr) of exposure to FBS- free media. Then TEX was enriched by a TEX isolation kit. The size, morphology, and protein content of isolated TEX were detected by DLS, FE-SEM, and BCA assay. The incorporation of miR-34a into the TEX was handled by a modified CaCl2 method. Confirmation of loading was assessed by real-time PCR. Then the cells were treated with 25 µg/ml of TEX or TEX-loaded with miR-34a for 48 hr and after staining with PI, cell cycle analysis was conducted. Then the effect of each treatment on the migration of 4T1 cells was tested by scratch test.

Results: Our results showed that 4T1 loading was fully successful. Treatment of 4T1 cells with TEX-miR-34a effectively arrested the progression of the cell cycle in the G0/G1 stage in comparison to treatment with either TEX or the untreated group. miR-34a- loaded TEXs reduced the migration ability of 4T1 cells up to 70% in proportion to the control group. TEX treatment exacerbated the 4T1 cells' power in migration toward untreated cells.

Conclusion: Our data revealed that 4T1-TEX could be considered a helpful biological carrier for miR-34a replacement therapy in containing the 4T1 cell progression and migration in the in vitro condition.

Keywords: Extra-cellular vesicles, PI, Triple-negative breast cancer

NK cell subsets and their functional molecules in the peripheral blood of patients with breast cancer

Reza Darvishvand[1], Somayeh Rezaeifard[2], Razieh Kiani[2], Sedigheh Tahmasbi[3], Zahra Faghih[2], Nasrollah Erfani[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran -Cancer Immunology and Immunotherapy group, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Cancer Immunology and Immunotherapy group, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Surgery, Shahid Faghihi Hospital, School of Medicine, Shiraz, Iran

Background: The study aimed to investigate NK subsets and their functional molecules in the peripheral blood of patients with breast cancer (BC) in comparison with healthy donors (HD).

Methods: Thirty untreated females with BC and 20 age-matched healthy women were enrolled in the study. Blood samples were collected and directly incubated with the adjusted concentration of fluorescent antibodies against CD3, CD56, CD16, CD27, CD11b, CD96, NKG2A, NKG2D, NKp44, CXCR3, perforin, and granzyme B. Red blood cells were then lysed using RBC lysis buffer, and the stained cells were acquired on a four-color flow cytometer.

Results: Our results indicated that the cytotoxic subset with CD3-CD56+CD27-CD11b+ phenotype was the most frequent subset (11.26% vs. 9.73% of lymphocyte population), while regulatory and tolerant subsets were relatively rare (less than 1%) in the peripheral blood of the patients and controls. Statistical analysis revealed that the mean expression of CXCR3 (based on MFI) in total NK cells (CD3-CD56+) and conventional cytotoxic subset (CD3-CD56dimCD16+) were significantly higher in the patients than controls (p=0.02 and p=0.03, respectively). On the other hand, the mean expression of granzyme B in conventional regulatory NK cells (CD3-CD56bright CD16-/+) and CD3- CD56- CD16+ NK cells, was lower in the patients than the corresponding subsets in healthy controls (0.03 and 0.004, respectively).

Conclusion: Higher expression of CXCR3 on NK cells in patients with BC might be associated with NK cell infiltration in response to higher expression of CXCL10 which has been shown to be expressed in the breast tumor microenvironment. However, lower expression of granzyme B in regulatory and CD3-CD56-CD16+ NK cells suggested a reduction of NK cell cytotoxic activity in patients with BC. This result might demonstrate an exhausted phenotype for NK cells in the blood of patients with BC.

Keywords: Breast cancer, Natural killer (NK) cells, NK cell subsets, Functional molecules

NK cell subsets in patients with acute myeloid leukemia (AML)

Zahra Faghih[1], Maral Mokhtari[2], Zahra Mansourabadi[3], Zohreh Bagheri[4], Sadaf Mohajerani[5]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
4. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
5. Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Natural killer (NK) cells, as the prototypic member of the innate immune system, are capable of direct killing tumor cells, and accordingly introduced as a biomarker for monitoring the immune system with prognostic value in a variety of cancers. These cells are recently subdivided into cytotoxic, regulatory, and tolerant subsets based on the function and relative expression of CD27 and CD11b markers. Here, we aimed to investigate the frequencies of these subsets in patients with acute myeloid leukemia (AML) malignancy in comparison to healthy individuals.

Methods: Fresh heparinized blood sample was obtained from 29 newly diagnosed patients with AML and 24 healthy donors. Whole blood was directly incubated with specific antibodies for CD56, CD16, CD27, and CD11b. After lysing red blood cells, the stained cells were acquired on a four-color FACSCalibur flow cytometer and the data were analyzed by FlowJo software version 10.8.1.

Results: NK cells, based on the expression of at least one of the CD16 and CD56 markers, accounted for 24.13% ± 30.22 (0.59 – 87.5) of lymphocyte/monocytes population in the patients with AML which was in the range of the control group (27.00 ± 9.86%). However, the frequency of total NK cells showed no significant differences, our analysis indicated that among different subsets, cytotoxic NK (CD16+CD56+CD11b+CD27+) was significantly more frequent among patients (50.70 ± 29.26) (p<0.001), while tolerant subset (CD16+CD56+CD11b-CD27-) was significantly dominant in healthy individuals (54.80 ± 29.95%) (p<0.05).

Conclusion: Our results collectively indicated that NK cells display heterogeneous populations with different functionality between normal and pathological conditions. We detected a more frequency of cytotoxic NK cells in patients while the tolerant subset was more frequent in controls, however, more studies in the larger population seem to be necessary for both prognostication and implication of these functional subsets in the field of immunotherapy.

Keywords: Acute myeloid leukemia, NK cells, Cytotoxic NK, Tolerant NK

Oncolytic Newcastle disease virus effects on immune response: a new issue in cancer treatment

Shabnam Bahrami[1], Ruhollah Dorostkar[2], Mahdieh Farzanehpour[2], Hadi Esmaeili Gouvarchinghaleh[2]

1. Department of Molecular Cell Biology and Genetics, Bushehr Branch, Islamic Azad University, Bushehr, Iran
2. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: Millions of people are diagnosed with cancer each year, and fighting it puts a heavy financial burden on communities and governments. Numerous advances have been made in the field of cancer, one of the newest methods is using oncolytic viruses. This study aimed to evaluate the effect of oncolytic Newcastle disease virus wild-type strains (NDV-WTS) on the immune system.

Methods: Forty mice were divided into 4 groups (10 animals in each group). The control group received PBS, and the experimental group 1 (NDV-WTS 1), Experimental group 2 (NDV-WTS 2), and experimental group 3 (NDV-WTS 3) received 101, 102, and 103 titers of Newcastle virus in 0, 14th and 28th days. On the 31st day, 100 µl of Newcastle virus was injected into the left footpads of animals. After 48 hours, Delayed-type hypersensitivity (DTH) reactions were measured. On the 33rd day, peritoneal macrophages were isolated. Then proliferation of the cells was measured by the MTT test. Neutral red uptake and respiratory burst of peritoneal macrophages were also assessed. Data were analyzed using SPSS v19.

Results: The results of the DTH test showed that footpad swelling in the control, NDV-WTS 1, NDV-WTS 2 and NDV-WTS 3 groups were 23. 51%, 23.55%, 23.62% and 23.57%. No significant differences were seen between the groups in this regard ($p > 0.05$). Negative nitro blue tetrazolium (NBT) test as the indicator of macrophage's respiratory burst, showed no significant difference between the groups ($p > 0.05$). The neutral red uptake assay and MTT test showed no significant differences between the groups ($p > 0.05$).

Conclusion: The results of this study showed that NDV-WTS in doses of 101, 102, and 103 have no adverse effects on healthy normal cells.

Keywords: Cancer, Virus, Newcastle virus, Immune system, Oncolytic virus, Mice

Overexpression of Wilms' Tumor 1 protein in the patients with Glioblastoma Multiform

Razie Kiani[1], Amir Reza Dehghanian[2], Ahmad Soltani[3], Somayeh Rezaeifard[4], Nasrollah Erfani[5]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Neurosurgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Cell Biology, Radboud University Medical Center/Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University Nijmegen, Netherlands
5. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Glioblastoma multiforme (GBM), a WHO Grade IV glioma is the most common primary brain tumor. Recently, the WT1 protein has been considered a new molecular target of cancer immunotherapy for several solid tumors. Using the RT-PCR method, our data demonstrated that the WT1 mRNA expression in the glioblastoma tissues was significantly higher than those in the medulloblastoma. The goal of the current study was to determine the expression of WT1 protein in tissue specimens' glioblastomas.

Methods: Immunohistochemistry (IHC) was used to examine WT1 protein expression in 30 surgical GBM specimens and 3 medulloblastomas (high grade (IV) childhood brain tumor; as negative control). Cases were classified according to IDH1 mutation status.

Results: Analyses indicated that IDH1wildtype is the most frequent in tumors among our cases (22 out of 30; 73.3%) and the remaining 5 cases (26.6%) were IDH1mutant. Through IHC, WT1 was overexpressed in 24 cases (86.7%), partially expressed in 4 cases (13.3%), and not expressed in only 2 cases. WT1 expression was absent in all control specimens. WT1 overexpression significantly differs between cases with wild-type and mutant IDH1 (p=0.048). This significance was not observed among IDH1mutant cases with partially expressed or overexpressed WT1 (p= 0.76).

Conclusion: These observations suggest that the WT1 gene might be closely associated with tumorigenesis of glioblastoma compared to medulloblastoma. Accordingly, WT1 peptide might be applicable as a potential target for immunotherapy of glioblastoma.

Keywords: Wilms' tumor gene 1 (WT1), Glioblastoma Multiforme (GBM), Medulloblastoma, K562

Programmed death Protein-1 blockade increases the cytotoxicity effect of Natural Killer Cells

Neda Parvini[1], Marzieh Ebrahimi[2], Fardin Fathi[1], AmirAli Hamidieh[3], AbbasAli Amini[1], Abbas HedayatiAsl[2], Zakaria Vahabzadeh[1]

1. Cellular and Molecular Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Pediatric Cell Therapy Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Programmed Death protein-1 (PD-1) is a surface receptor known as an immunological checkpoint inhibitor for immune cells such as Natural killer (NK) cells. NK cells are promising approaches in treating blood malignancy. Inhibitory checkpoints play an important role in the exhaustion of the cytotoxicity function of NK cells in patients. The aim of this study was to evaluate the PD-1 blockade on the NK cell's cytotoxicity effect against the K562 cell line as a gold standard cell line.

Methods: CD56+ 16+ NK cells were isolated from healthy donor PBMC by BD FACS Aria II and stimulated overnight by IL-15 (10 ng/ml). Then, PD-1 checkpoints were blocked by Keytroda (Anti-PD-1 monoclonal antibody). In order to cytotoxicity effect evaluation, human CML cell-line K562 was plated in 48-well plates at 1 × 105 cells/well in triplicate as target cells. Activated NK cells as the effector cells were co-cultured with K562 cells at an effector: target (E: T) ratios of 1:1, 5:1, and 10:1 followed by incubation for 6, 12, and 48 h at 37°C in 5%CO2. Then, the cytotoxicity of NK cells was evaluated using Propidium iodide (PI) staining by flow cytometry.

Results: The purity of NK cells was 95.07 ± 4.89 after cell sorting. The cytotoxicity of activated NK cells against K562 cell lines without PD-1 blockade (control group) and PD-1 blockade in a group with a 10:1 E: T ratio after 12 h incubation was 20.15%; SD:1.508 and 51.975: SD:4.136, respectively. Statistical analysis showed a significant increase in the cytotoxicity function of PD-1 blockade-activated NK cells compared to the control group.

Conclusion: In conclusion, PD-1 inhibiting on the surface of NK cells results in increasing the anti-tumor function of NK cells. It was shown that the cytotoxicity function of activated NK cells against the K562 cell line is higher than the control group. We suggest that PD-1 blockade on the NK cells can be a hopeful option in treating blood malignancy. However, confirmation of this result requires more studies.

Keywords: PD-1 inhibiting, NK cells, Cytotoxicity effect, K562

Prostate cancer cells-derived exosomes promote endothelial to mesenchymal transition (EndMT) in the HUVEC cell line

Sepideh Sohrabi[1], Maryam Mohammad hassani[1], Sepideh Maralbashi[1], Fatemeh Naderali[1], Tohid Kazemi[1]

1. Department of Medical Faculty of Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Cancer cells promote angiogenesis and metastasis processes using several mechanisms. Of them, secreting exosomes has been recently found to have important roles. In this study, the expression of endothelial and mesenchymal markers was investigated in human umbilical vein endothelial cells (HUVECs) after treatment with exosomes derived from prostate cancer (PC) cell lines.

Methods: PC3 and DU145 cell lines were cultured under normoxia and hypoxia conditions for 24 hours. Then, secreted exosomes were isolated via ultracentrifuge and verified by transmission electronic microscope (TEM). The HUVEC cell line was treated with various concentrations of exosomes for 24 hours and proliferation of cells was examined by MTT assay. Expression of markers associated with endothelial and mesenchymal features including CD31, VE-cadherin, MMP-9, MMP-2, N-cadherin, α-SMA, and vimentin in HUVEC were evaluated by quantitative real-time PCR.

Results: Findings revealed that exosomes derived from prostate cancer cells grown under normoxic conditions significantly down-regulated the expression of endothelial markers including CD31 and VE-cadherin. Additionally, expression of mesenchymal markers including MMP-9, MMP-2, N-cadherin, vimentin, and a-SMA was down-regulated by exosomes derived from PC cell lines grown in both conditions.

Conclusion: Results of this study showed a possible mechanism for tumor-promoting features of exosomes derived from cancer cells via augmenting the EndMT process in human endothelial cells.

Keywords: exosomes, prostate cancer, endothelial to mesenchymal transition, hypoxia.

Protective potential of piroxicam on human peripheral blood mononuclear cells against the suppressive capacity of glioblastoma cell lines

Jahangir Abdesheikhi[1], Farnaz Sedghy[2]

1. Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

Background: Dexamethasone, a common medication used in the treatment regimen of glioblastoma, has broad inhibitory effects on immune responses. Here, in an in vitro study, we examined the effects of piroxicam, a potent substitute for dexamethasone, on peripheral blood mononuclear cells (PBMCs) co-cultured with two glioblastoma cell lines, U-87 MG and A-172 cells.

Methods: MTT assay was used to determine the proliferation of PBMCs treated with piroxicam, or dexamethasone. In addition, to evaluate the effects of drugs on the cell cycle distribution, DNA content per cell was analyzed in PBMCs and A-172 cell lines using flow cytometry. Oxidative parameters, including superoxide dismutase-3 (SOD3) activity and total anti-antioxidant capacity, lactate dehydrogenase (LDH) activity, as well as IFN-γ and TGF-β levels were measured in PBMCs alone or in the presence of cell lines using ELISA.

Results: Unlike dexamethasone, piroxicam showed a protective effect on PBMCs against both glioblastoma cell lines. Furthermore, while dexamethasone reduced the proliferation of PBMCs, piroxicam had no adverse effect on the proliferation. Cell cycle analysis showed a reduction in the G2/M phase in piroxicam-treated A-172 cells. Additionally, dexamethasone limited the cell cycle progression by increasing the fraction of PBMCs in G0/G1. Interestingly, after co-culturing piroxicam-treated PBMCs with cell lines, a remarkable rise in the LDH activity was observed. Although not significant, piroxicam partially decreased TGF-β levels in both cell lines.

Conclusion: Our findings suggested a protective effect of piroxicam, but not dexamethasone, on PBMCs against inhibitory mechanisms of two glioblastoma cell lines, U-87 and A-172 cells.

Keywords: Glioblastoma, Piroxicam, peripheral blood mononuclear cells (PBMCs), Dexamethasone, U-87 MG and A-172

Relative expression of CD112 and BATF in PBMC of patients with chronic lymphocytic leukemia

Ahmad Najafi[1], Matineh Sabaghi-Rostami[1], Hossein Asgarian-Omran[2], Reza Valadan[3], Ramin Shekarriz[4], Ehsan Zaboli[4], Armin Akbar[1], Ghasem Janbabaei[4], Mohsen Tehrani[5]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Immunology and Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
3. Molecular and Cell-Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran
4. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran
5. Department of Immunology and Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

Background: BATF, as a transcription factor, and CD112, as a receptor for TIGIT, are involved in T-cell exhaustion. We investigated BATF and CD112 gene expression in the peripheral blood mononuclear cells of CLL patients and healthy subjects.

Methods: In a case-control study, 33 patients with CLL and 20 sex- and age-matched healthy individuals were enrolled. Diagnosis and classification of patients were done according to immunophenotyping via flow cytometry and RAI staging system, respectively. Relative mRNA expression of BATF and CD112 was measured using qRT-PCR.

Results: Our results showed that the expression of BATF and CD112 in CLL samples was significantly decreased in comparison to those of the healthy controls (p=0.0236 and p= 0.0002, respectively).

Conclusion: These findings suggest that BATF and CD112 not only play a role in T cell exhaustion but also might influence on effector differentiation program in CLL, which warrants further study in the future.

Keywords: Chronic lymphocytic leukemia, BATF, CD112, T-cell exhaustion, immune checkpoint blockade

Role of microRNA-124/EZH2 axis in EBV-infected Gastric cancer

Nasir Arefinia[1], Amir Sasan Mozafari[2]

1. Jiroft University of Medical Sciences, Jiroft, Iran

Background: microRNA (miR)-124, miR-21, and miR-494 are involved in regulating various cellular processes as tumor suppressors. In this study, target miR methylation levels in the 304 pairs of GC (and corresponding non-tumor tissues) were assessed.

Methods: To detect EBV and H. Pylori DNA in GC tissues, a commercial real-time PCR EBV and H. Pylori kit was used. Following the discovery of hyper-methylation of the miR-124 gene promoter, its expression level was assessed by qPCR. Enhancer of zest homolog 2 (EZH2) is identified as a target of miR-124 by bioinformatics analysis. QPCR was also applied to verify the interaction between miR-124 and EZH2.

Results: Of the 304 subjects, EBV and H. Pylori DNA were detected in 9.5% and 15.1% of GC patients, respectively. We also found significant differences in the miR-124 methylation levels of EBV-infected GC patients compare to total GC patients, H. pylori-infected GC patients, GC patients with no EBV and H. pylori-infected, and non-tumor tissue. Bioinformatics analysis and qPCR assays showed that EZH2 was a target gene of miR-124 and was negatively correlated with the level of miR-124 in cancer tissues. Our study suggested that the miR-124 gene promoter was hyper-methylated, and its expression was significantly down-regulated in EBV-infected GC tissues.

Cunclusion: miR-124 binding sites were present in the 3′-UTR of the EZH2 gene and its expression was up-regulated so, miR-124 was a tumor suppressor in EBV-infected GC, and miR-124 may associate with EBV-infected GC via targeting EZH2.

Keywords: Gastric cancer, Epstein Bar Virus, miR-124, Helicobacter pylori, Methylation, miR-494, miR-21, EBV, H. pylori, EZH2.

Silencing V-set and immunoglobulin domain containing 3 (VSIG-3) reduced cell viability in the A2058 melanoma cell line

Najibeh Shekari[1], Dariush Shanehbandi[2], Seyed Amir Jalali[1], Behzad Baradaran[3]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Cancer immunotherapy has taken on a special place in oncology through the discovery of immune checkpoints and the development of inhibitors to combat their effects. Although V-set and immunoglobulin domain containing 3 (VSIG-3) has been introduced as a noteworthy ligand for V-domain immunoglobulin suppressor of T cell activation (VISTA), a special immune checkpoint, still little is known about VSIG-3's role in tumorigenesis. Hence, we aimed to evaluate the possible role of VSIG-3 on the viability of melanoma cell lines.

Methods: Different melanoma cell lines (HFFF2, A375, SK-MEL-3, and A2058) were evaluated by RT-PCR regarding the expression of VSIG-3. Using the different concentration of VSIG-3-siRNA, the role of VSIG-3 in the viability of the A2058 cell line were assessed via MTT assay.

Results: Statistical analysis showed that among melanoma cell lines, A2058 has the highest expression rate of VSIG-3 ($p<0.001$), and was chosen as the target cell line. Knocking down of VSIG-3 was conducted by electroporation and VSIG-3 expression was decreased significantly in A2058 cells ($p<0.001$). MTT assay showed that VSIG-3-siRNA decreased the viability of A2058 cells significantly ($p<0.01$).

Conclusion: Decreased viability of A2058 cells indicated that VSIG-3 has a role in the regulation of the viability of melanoma cells. Furthermore, combining this finding with the fact that inhibiting the VISTA/VSIG-3 pathway increases anticancer effects, VSIG-3 appears to hold great promise as a therapeutic target for cancer.

Keywords: Immune checkpoint, VISTA, VSIG-3, Melanoma, Viability

Simultaneous expression of PD-1 and PD-L1 in peripheral and central immune cells and tumor cells in the benign and malignant salivary gland tumors microenvironment

Sajjad Gerdabi[1], Nasrollah Erfani[1]

1. Department of Immunology, School Medicine, Shiraz University of MedicaScienceses, Shiraz, Iran

Background: To investigate the differential expression of PD-1 and PD-L1 in salivary gland tumors (SGTs, malignant and benign subtypes) and determine their association with the clinicopathological characterization of the patients.

Methods: Immunohistochemistry was used to examine PD-1 and PD-L1 expression in specimens from 83 patients with primary SGTs including salivary ductal carcinoma (SDC), adenoid cystic carcinoma (AdCC), acinic cell carcinoma (ACC), mucoepidermoid carcinoma (MEC), war thin's tumors (WT), pleomorphic adenoma (PA) and other subtypes.

Results: The expression of PD-1 in peripheral and central immune cells (ICs) of MEC, and peripheral ICs of ACC was significantly higher than those with AdCC (p=0.02, *p*=0.02, *p*=0.03, respectively). Interestingly, the expression of PD-1 was also observed in peripheral and central malignant tumor cells (TCs), particularly in SDC and ACC. Despite no significant difference in PD-L1 expression of TCs among malignant subtypes, the peripheral and central ICs of ACC and MEC were revealed to express PDL-1 significantly more than those with AdCC ($P<0.05$). WTs were rich in PD-1/PD-L1 expressing ICs. However, the tumor microenvironment of PA generally had low levels of PD-1/PD-L1 expression. In general, the expression of PD-1 in peripheral and central TCs was found to be significantly higher in malignant tumors than in benign ones (P=0.002 and P=0.003, respectively).

Conclusion: The simultaneous presentation of PD-1 and PD-L1 in TCs and ICs of SGTs, their significant association with disease severity as well as the positive correlation between these immune checkpoints may suggest the therapeutic potential of anti-PD-1 and anti-PDL-1 combinational immunotherapy for SGTs.

Keywords: Salivary gland tumor, Immunohistochemistry, Immune checkpoint, PD-1, PD-L1

siRNA mediated LncRNA PVT1 knockdown inhibits Paclitaxel-induced inflammatory cytokines expression in AGS human gastric adenocarcinoma cells

Bahar Naseri[1], Javad Masoumi[2], Dariush Shanehbandi[3]

1. Tabriz University of Medical Sciences, Tabriz, Iran

Background: Tumor milieu inflammation has been known as a potent tumor progression factor in gastric cancer and can be induced by various chemotherapeutic agents including Paclitaxel. Previous studies have revealed that LncRNA PVT1 expression has been elevated in gastric cancer tissues and cell lines and is positively correlated with tumor progression in patients. Furthermore, it has been reported that LncRNA PVT1 could mediate inflammation in various conditions in vitro and in vivo. The present study aimed to explore the effect of PVT1-specific siRNA transfection in combination with paclitaxel treatment on the expression of some inflammatory cytokines in gastric cancer AGS cells in vitro.

Methods: AGS cells were cultured in RPMI-1640 medium and then transfected with 100 nM siRNA PVT1 (siPVT1) to analyze the silencing efficiency by qRT-PCR assay 48 h after transfection. Cultured cells were then transfected with siPVT1 and/or treated with 20 µg/ml (IC50 value) Paclitaxel and incubated for 24h at 37Ċ and CO2 5% conditions. Synthesized cDNAs from extracted RNAs were used to evaluate the expression of IL-6, IL-10, IL-17, TNF-α, and IFN-γ genes by qRT-PCR technique. Finally, the obtained data were analyzed using appropriate statistical tests.

Results: siPVT1 transfection could significantly reduce the expression of lncRNA PVT1 in AGS cells ($p< 0.0001$). Compared with the control group, Paclitaxel treatment significantly increased the expression of TNF-α ($p < 0.05$), IL-6, IL-10, IL-17, and IFN-γ genes ($p < 0.001$). In comparison with the Paclitaxel group, siPVT1, and siPVT1+ Paclitaxel groups significantly decreased the expression of IL-6, IL-10 ($p < 0.0001$), IL-17, TNF-α, and IFN-γ genes ($p < 0.001$). Except for IL-6 and TNF-α genes ($p < 0.05$), there were no significant differences in the expression of other genes between the control group and siPVT1 and siPVT1+ Paclitaxel groups.

Conclusion: Our results showed that PVT1 knockdown could significantly inhibit the Paclitaxel-induced inflammation in gastric cancer cells and can be considered as a potential therapeutic target in combination with other anti-cancer therapies including chemotherapy.

Keywords: Gastric cancer, LncRNA PVT1, Paclitaxel, Inflammation, Cytokine

Synthesis, characterization, and both in vivo and in vitro toxicity assessment of new cobalt Schiff base complex in breast cancer mouse model; Theoretical calculations

Fatemeh ghorbani[1]

1. Department of Immunology, Faculty of Medical Sciences Semnan University, Semnan, Iran.

Background: Increasing cancer drug chemo-resistance, especially in the treatment of breast cancer, alarms the immediate need for newer and effective anticancer drugs. Until now, chemotherapeutics based on metal complexes are considered the most effective treatment modality. Therefore, investigators seek new alternatives to cisplatin that may be more effective and/or safer. The purpose of this research is to synthesize an efficient none platinum chemotherapy drug and its therapeutic effect evaluation on a tumor-bearing mice model.

Methods: In the present work, new transition metal complexes derived from Schiff base ligands were synthesized and characterized by elemental analyses, FT-IR, and UV-Vis spectroscopy. MDA-MB231, 4T1 was cultured and DPPH free radical, cytotoxic effect, migration assay, and apoptosis induction were investigated. Further, three doses of the s ofhavemplex have been administered to female Balb/c 4T1-tumor bearing mice, and tumor growth, mice weight, splenocyte proliferation index, and cytokines were done for further analysis.

Results: The results revealed that Co (III) complexes exhibited considerable cytotoxic activity against MDA-MB231 and 4T1 cell lines. Complex C treatment leads to cancer cell apoptosis and a profound inhibition effect on the migration of MDA-MB231 cells. The molecular docking test confirmed the binding of the complex C with the most stable state, so interaction with the DNA minor groove finally induce apoptosis. Our results demonstrated that complex C significantly inhibited mouse breast cancer growth, and boosted IFN-γ, TNF-α secretion, and significant decrease in the level of IL-4, and IL-1β in response to stimulation by tumor lysate.

Conclusion: Our results revealed that complex C has a lethal effect on malignant breast cancer cells by stimulating the apoptosis induction pathway, and in a mouse model, it reduces and inhibits tumor growth by induction of apoptosis and stimulating the mouse immune system accompanied by alteration of cytokine.

Keywords: Breast cancer; Schiff base complex; Cytotoxicity; Apoptosis; Cell migration

Texosomes Enriched By MicroRNA-34a Inhibit Inflammation-Induced Proliferation and Migration of Colorectal Cancer Cells

Maryam Hosseini[1], Kaveh Baghaei[2], Masoumeh Ebtekar[3], Davar Amani[4]

1. Trauma Research Center, Shahid Rajaee (Emtiaz) Trauma Hospital, Shiraz University of Medical Sciences, Shiraz, Iran
2. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
4. Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: MicroRNA (miR)-34a, as a main tumor suppressor in several malignancies, modulates multiple genes associated with tumor proliferation, invasion, and progression through chronic inflammation. However, introducing an effective nanoparticle to carry microRNAs is challenging. Tumor-derived exosomes known as exosomes are novel nanoparticles found in recent research. Thus, we investigated how exosomes delivering miR-34a (TEX-miR-34a) affect colorectal cell (CRC) progression in vitro.

Methods: Texosomes (TEXs) were purified from the starved CT-26 murine CRCs and enriched by miR-34a using the calcium chloride (Cacl2) modified solution. Subsequent to the detection of miR-34a expression in the enriched TEXs, the proliferation rate of CT-26 cells treated by multiplicity concentrations of either TEXs or TEX-miR-34a was examined. Furthermore, the apoptosis and migration of the cells subjected to both TEX-miR-34a and TEX were measured. Thereafter, the expressions of miR-34a target genes such as IL-6R, STAT3, PD-L1, and VEGF-A were determined in the treated cells.

Results: The proliferation rate of CT-26 cells was restricted following the treatment with TEX-miR-34a and the apoptosis levels of the cells also enhanced dose-dependently. TEX-miR-34a was able to diminish the migration rate of the TEX-miR-34a treated cells and the expressions of IL-6R, STAT3, PD-L1, and VEGF-A genes were significantly reduced. Moreover, TEXs alone increased the apoptosis rate of tumor cells and repressed the proliferation and migration of these cells.

Conclusion: Texosomes isolated from the starved CT-26 cells were capable of properly delivering miR-34a into tumor cells with high functionality maintenance for miR-34a to modulate genes related to tumor progression via induction inflammatory state alongside no positive effect of TEXs favoring cancer cells. These results suggest TEX-miR-34a as a favorable adjuvant in CRC therapy.

Keywords: Texosome, Colorectal cancer, microRNA-34a, Inflammation

The Additive Cytotoxic Effect of Exosomes Derived From Natural Killer Cells with Mitoxantrone in Liver Cancer Treatment

Zahra Sadat Hashemi[1], Benyamin Baghani[2], Mahdieh Shokrollahi Barough[3], Ardeshir Ghavamzadeh[4], Ramin Sarrami Forooshani[5]

1. ATMP Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran

Background: Primary liver cancer, mainly hepatocellular carcinoma, remains difficult cancer to treat and is one of the most common cancers worldwide with high mortality. Exosomes are nanovesicles released by almost all cells in the body. Previous studies have shown that NK cells release exosomes in both resting and activated states. We previously found that NK cell-derived exosomes express lethal proteins [ie, Fas ligand (FasL) and perforin] and inhibit cancer growth. This suggests that NK cell-derived exosomes exhibit effective immunological functions even in the absence of specific stimuli. Mitoxantrone is used to treat certain types of cancer. This is a DNA-reactive agent that intercalates into deoxyribonucleic acid (DNA) through hydrogen bonding, causing crosslinks and strand breaks.

Methods: NK cells were obtained from peripheral blood mononuclear cells (PBMC) and NK-Exos were isolated from NK cell expansion media. The HepG2 cell line was cultured as liver cancer cells in high glucose DMEM with 10% FBS. 104 cells/100µl were seeded in 96-well plates and after the cells reached 70% confluence, the wells were treated by NK-Exos, Mitoxantrone, and both of them. The cytotoxic effect of Mitoxantrone/Exo-NK in human liver cancer was investigated by MTT, apoptosis, scratch and migration assay, and colony formation.

Results: All the viability tests also showed that the drug and Exos were more effective when used together than when used alone. Apoptosis experiments also showed the movement of cells toward death and the induction of apoptosis in liver cancer cells.

Conclusion: Mitoxantrone/Exo-NK showed a potent inhibitory effect on proliferation and induced apoptosis in human liver cancer. The use of exosomes in combination with other therapies, especially chemotherapy, to improve outcomes and promote survival in patients is a promising avenue for future studies.

Keywords: NK cells, Exosomes, Mitoxantrone, Liver cancer

The anti-cancer effect of pomegranate extract on MCF 7 cell line

Nafiseh Paysokhan[1], Arefeh Majidi khameneh[2], Zahra Matluobi[3]

1. Student Research Committee, Sabzevar University of Medical Sciences, Sabzevar, Iran
2. Cellular and Molecular Research Center Affiliated to Sabzevar University of Medical Science

Background: The advent of resistant cancer cells and the severe side effects of chemotherapy drugs have led scientists to find solutions to these problems. Today, one of the solutions that has been considered is the use of herbal extracts along with chemotherapy to reduce the side effects of chemotherapy drugs and at the same time increase their anti-cancer effects. One of these candidate herbs is pomegranate. Pomegranate extract contains ellagitannin, which is a precursor of ellagic acid. Ellagitannin has strong antioxidant properties that can induce apoptosis. Unlike ellagic acid, ellagitannin is soluble. In this study, we decided to test pomegranate extract on MCF 7 cell line.

Methods: The MCF7 cells were cultured in DMEM, supplemented with 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. Cells were maintained in a humidified incubator at 37°C with 5% CO2.the next, cells were seeded in the plate. then Cells were exposed to various concentrations of pre-prepared pomegranate extract that was sterilized by filtration. After 24 hours, the toxicity level was measured by MTT assay. Also, this test was performed on lymphocyte cells, to evaluate the toxicity of extract on normal cells.

Results: The results of the MTT test showed that a concentration of pomegranate extract that caused the death of MCF 7 cells had no cytotoxicity effect on lymphocyte cells. The death rate of MCF 7 cells in 500, 250, and 100 µl of pomegranate extract was 76%, 45%, and 24% respectively.

Conclusion: It seems that pomegranate extract could be used as a complementary drug besides chemotherapy, although the confirmation of these findings requires further research on other cancer cells.

Keywords: pomegranate extract, MCF 7, cancer, ellagitannin

The assessment of IL-10 and IL-19 gene expression levels in breast cancer tumor-draining lymph nodes

Atri Ghods[1], Elham Babaeinia[2], Reza Rasolmali[3], Abdoul-Rasoul Talei[3], Fereshteh Mehdipour[1], Abbas Ghaderi[4]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Pathology, Shiraz Central Hospital, Shiraz, Iran
3. Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: IL-10 and IL-19 are two members of the IL-10 family with both pro- and anti-tumor activities in different solid tumors. They are produced by different cell type. We designed this study to determine the gene expressions of IL-10 and IL-19 in mononuclear cells obtained from breast cancer tumor-draining lymph nodes (TDLNs) and assess the association of their expression levels with the clinicopathological characteristics of the patients.

Methods: lymph node samples were obtained from 69 patients with breast cancer. Mononuclear cells were isolated, and then the gene expression of IL-10 and IL-19 was determined by using the Real time-PCR technique.

Results: The expression of IL-10 was not different in patients with breast cancer with different stages, grades, nodal and hormone receptors (ER, PR, and HER2) status, and tumor size; however, the IL-19 expression was significantly lower in patients with grade III in comparison with grade I+II (p=0.03). Moreover, based on the mean age of the participants (49.5 y/o), we categorized them into two groups: younger patients who were ≤ 50 y/o and older patients who were > 50 y/o. Analysis revealed that the expression of IL-19 was significantly lower in younger patients with grade III compared with grade I+II (p=0.001). Moreover, among younger LN+ patients, the expression of IL-19 was significantly lower in N2 compared with N1 and N3 nodal status (p=0.013 and p=0.003, respectively).

Conclusion: The IL-10 expression was not associated with clinic-pathological parameters; but, the expression of IL-19 was significantly decreased in higher grades and more lymph node involvement.

Keywords: Breast cancer, Tumor draining lymph node, Gene expression, IL-10, IL-19

The association between allergy and cancer: new perspectives

Parnian Jamshidi[1], Narjes Mosavari[2], Donya Najafi[3], Mohammad Amin Siri[4], Noosha Samieefar[4], Nima Rezaei[5]

1. School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Medical Science and Technology Department, Science and Research Branch of Islamic Azad University, Tehran, Iran
4. Student Research Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Allergies and cancer are different medical conditions studied extensively in recent years. While allergies are caused by an overactive immune system reacting to harmless substances, cancer is a group of diseases that occur when cells in the body grow uncontrollably. Although the two conditions may seem unrelated, recent research suggests a potential association between them.

Methods: We performed a search in PubMed and Embase databases with the following keywords: "allergy" or "hypersensitivity" and "cancer" or "malignancy" or "neoplasm".

Results: Several studies have shown that individuals with allergies may have a lower risk of developing certain types of cancer, such as acute and chronic leukemia, esophageal, stomach, cervical, and pancreatic cancer, melanoma, and non-melanoma skin cancer, and malignant brain tumors like glioma. This protective effect is thought to be due to the immune system's ability to recognize and destroy cancer cells. However, some studies have also found an increased risk of certain types of cancer in individuals with allergies, including lung cancer and squamous cell carcinoma of the skin.

Conclusion: The mechanisms behind the association between allergies and cancer are not yet fully understood, and the current literature is inconclusive. Immune surveillance hypotheses and antigenic stimulation theory are two main contradictory hypotheses about this linkage. Further research is required to delineate this issue.

Keywords: Allergy, Cancer, Hypersensitivity, Malignancy, Neoplasm

The association between CD3+ and CD8+tumor-infiltrating lymphocytes (TILs) and prognosis in patients with pancreatic adenocarcinoma

Mahshid Panahi[1], Fereshteh Rezagholizadeh[2], Shabnam Mollazadehghomi[3], Pooya Farhangnia[4], Mohammad Hadi Karbalaie Niya[1], Hossein Ajdarkosh[1], Fahimeh Safarnezhad Tameshkel[1], Seyed Mohammad Heshmati[1]

1. Gastrointestinal and Liver Diseases Research Center, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Molecular and Cellular Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Immunology Board for Transplantation and Cell-Based Therapeutics (ImmunoTACT), Universal Scientific Education and Research Network (USERN), Tehran, Iran
4. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Pancreatic adenocarcinoma (PDAC), with more than 250,000 deaths each year, is the eighth leading cause of death worldwide, with a five-year survival of less than 5% and a median recurrence time between 5 and 23 months. The association between PDAC and CD3+/CD8+ tumor-infiltrating lymphocytes (TILs) and the extent of tumor spread and clinical outcomes has been recently shown. This study aimed to determine and compare the density of TILs and their association with disease prognosis in patients with PDAC.

Methods: In this study, we collected PDAC tissues and corresponding adjacent normal tissues from 64 patients with TIL-positive PDAC. The immunohistochemistry method was used for the detection of the expression levels of CD3+ and CD8+ TILs in PDAC tissues. Also, the completed follow-up history was evaluated for at least five years.

Results: The frequency of intratumoral and peritumoral TILs was 20 (31.2%) and 44 (68.8%), respectively. The mean density of CD3+ TILs and CD8+ TILs was 67.73%±20.17% and 69.45%±17.82%, respectively. The density of CD3+ TILs and CD8+ TILs was not associated with overall survival nor metastasis-free survival of the patients and tumor grade. However, the density of TILs was significantly lower in those patients who experienced tumor recurrence than those without this recurrence.

Conclusion: TILs density was high in patients with PDAC. The density of both CD3+ and CD8+ TILs was significantly lower in patients who experienced tumor recurrence. Thus, this study suggests that tracking and determining the density of CD3+ and CD8+ TILs might be effective in predicting PDAC recurrence.

Keywords: Tumor-infiltrating lymphocyte, TIL, PDAC, Pancreatic ductal adenocarcinoma, CD8+ TIL

The association of LPPR4/PRG1 expression levels in patients with Prostatic cancer

Nazanin Nazari[1], Asma Nikkhouy Tanha[2], Mahsa Talebi[3], Elham Nadimi[4], Banafsheh Rastegari[5], Golnoosh Sharafieh[6]

1. Immunology Department, Shiraz University of Medical Sciences, Shiraz, Iran
2. Tehran University of Medical Sciences, Tehran, Iran
3. Department of Plant Protection, College of Agricultural Biotechnology, Shahed University, Tehran, Iran
4. Tehran University of Medical Sciences, Tehran, Iran
5. Diagnostic Laboratory Science and Technology Center, Shiraz University of Medical Sciences, Shiraz, Iran
6. Shahed University, Tehran, Iran

Background: Prostate cancer is considered the most abundant cancer among mail with the second level of mortality worldwide. The increasing rate of prostate cancer in the Middle Eastern population has driven the development of novel prognostic biomarkers. The aim of the present study was to investigate the lipid phosphate phosphatase-related protein type 4 expression levels in patients with prostate cancer.

Methods: The expression profile of the lipid phosphate phosphatase-related protein type 4 gene was assessed using TaqMan quantitative Real-time PCR. A total number of 40 cancer and 41 normal prostate biopsies were examined during Feb-Dec 2019. Several interrupting factors like distribution, family history, age, and smoking habits were considered and the expression levels in the cancer stage were analyzed with the help of Kolmogorov–Smirnov, Chi-squared, and Mann-Whitney, respectively.

Results: There was no significant correlation between lipid phosphate phosphatase-related protein type 4 expression levels with prostatic neoplasm out of control (p=0.221). Moreover, there was no significant relation between lipid phosphate phosphatase-related protein type 4 and cancer stages. While familial history had a positive effect on cancer development (p=0.001), smoking showed no significant effects (p=0.097).

Conclusion: The present results indicated that the lipid phosphate phosphatase-related protein type 4 contributed as a risk factor in prostatic neoplasm prognosis in patients with a family history.

Keywords: Prostate cancer, LPPR4/PRG1, Prognosis

The changed phenotypic properties of monocyte-derived dendritic cells in patients with prostate cancer

Parisa Bakhshi[1], Maryam Nourizadeh[2], Laleh Sharifi[3], Monireh Mohsenzadegan[4], Mohamad Farajolahi[5]

1. Department of Medical Biotechnology, School of Allied Medical Sciences, Iran University of Medical Sciences
2. Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran
3. Uro-Oncology Research Center, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Iran University of Medical Science, Tehran, Iran
5. Department of Medical Biotechnology, School of Allied Medical Sciences, Iran University of Medical Sciences

Background: Dendritic cells (DCs) play a pivotal role in anti-tumor immune responses. Although clinical trial outcomes have shown limited success, a plethora of research is in progress on monocyte-derived DCs (Mo-DCs) cancer vaccines.

Methods: Here, we generated Mo-DCs from prostate cancer patients (PCa-DCs) and phenotypically compared them with healthy donors' DCs (HD-DCs).

Results: PCa samples had significantly lower monocyte count and CD14 expression than healthy samples (p <0.0001). Additionally, PCa-DCs expressed significantly lower levels of maturation markers like HLA-DR, CD80, and CD86 than HD-DCs (p=0.123, p=0.884, and p=0.309, respectively).

Conclusion: These impaired phenotypic characteristics could be attributed to impaired functionality and cause the limited success of DC vaccines. Thus, there is a need to investigate alternative processes for developing Mo-DC characteristics.

Keywords: Co-stimulatory molecules, dendritic cells, Prostate cancer

The Cytotoxic Effect of Natural Killer Cells Derived Extracellular Vesicles (NK-EVs) on pancreatic cancer spheroids

Marzieh Ebrahimi[1], Soroor Eslahi[1], Hamid Asadzadeh[2], Faezeh shekari[1], Melika Sheykhan[1], alireza khosravani[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Iran
2. Department of Adult Gastroenterology and Hepatology, School of Medicine University of Shahid Beheshti University of Medical Sciences

Background: Being a progressive malignancy, pancreatic cancer resists traditional treatments. Dendritic cells, natural killer cells, and T cells can significantly enhance the efficacy of current therapeutic approaches. However, they are inhibited in the cancer's microenvironment. Natural killer (NK) cell-secreted nanoparticles, also known as extracellular vehicles (EVs), are currently being studied for potential use in cancer therapy due to their apparent stability against the tumor microenvironment. This study examines the cytotoxicity of NK-EV fractions on pancreatic cancer spheroid models.

Methods: NK cells were cultured and centrifuged at 2000g (group A), 10000g (group B), and 20000g (group c). The NK-EVs were characterized using SEM and DLS techniques. Specific markers of NK-EVs were identified using Western blot. Various concentrations of NK-EVs were administered to spheroids obtained from the Panc-1 cell line, and the cytotoxicity of NK-EVs was assessed using flow cytometry and the MTS assay.

Results: DLS data showed that group A EVs were 1020 nm, group B 548 nm, and group C 352 nm. Western blot confirmed that NK-EVs expressed CD63 and TSG101. In group C, the SEM showed EV-like vesicles that were oval and approximately the same size. These groups of EVs were treated to spheres produced, ranging from 300 nm to 450 nm. MTS results showed that group C EVs have the maximum cytotoxicity (IC50: 17.77 ±0.83 µg/ml, $p< 0.003$). MTS results were confirmed by flow cytometry analysis.

Conclusion: We suggest that EVs, produced by NK cells, have cytotoxic effects on pancreatic cancer cells. It has the potential as a novel anticancer agent for the treatment of human pancreatic cancer and can boost NK cell activity.

Keywords: PANC-1, Pancreatic cancer, Natural Killer Cells, Extracellular vesicles, NK-EVs, spheroids

The effect of adipose-tissue derived mesenchymal stem cells on proliferation, apoptosis, and cytokine expression of the healthy individual's PBMCs co-cultured with HeLa cell line

Maryam Dorfaki[1], Fatemeh Faraji[2], Mahdi Ghatreh Samani[3]

1. Department of Microbiology and Immunology, School of Medicine, Shehrekord University of Medicine Sciences, Shahrekord, Iran
2. Antimicrobial Resistance Research Center, Institute of Immunology and Infection Diseases Iran University of Medicine Sciences, Tehran, Iran
3. Department of Microbiology and Immunology, School of Medicine, Shehrekord University of Medicine Sciences, Shahrekord, Iran

Background: Cervical cancer is one of the most common cancers in women. The immune system plays an important role in controlling tumor growth. Mesenchymal stem cells (MSC) have been used in clinical trials to treat many diseases. The aim of this study is to investigate the effect of adipose tissue mesenchymal stem cells on the proliferation, apoptosis, and cytokine expression of (peripheral blood mononuclear cells) PBMCs of healthy individuals in co-culture with cervical cancer cells HeLa.

Methods: MSCs were isolated using collagenase I and then confirmed. The conditioned medium was obtained from the culture of MSCs for 1 to 5 days. The effect of condition medium and MSCs on proliferation, apoptosis, and gene expression of cytokines including TNF-α, TGF-β, IL-4, IL-2, and IFN-γ in PBMCs was investigated in monoculture and co_culture with HeLa cells. CFSE (Carboxyfluorescein succinimidyl ester) kit, Annexin V/PI kit, and Real-time PCR method were used to investigate proliferation, apoptosis, and gene expression, respectively.

Results: The results showed that treatment with the conditioned medium collected from MSC culture after 3 days for 48 hours led to a significant increase in the proliferation of PBMCs in monoculture and co-culture with HeLa cells and significantly decreased the proliferation of HeLa cells. There was no effect on the apoptosis of PBMCs, but the apoptosis of HeLa cells increased significantly. The gene expression of cytokines IL2, INF-γ, and TGF-β in PBMCs was significantly increased due to treatment with condition medium in the co-culture of PBMCs and HeLa cells. The presence of MSCs significantly increased the proliferation of PBMCs in single and co-culture with HeLa cells for 24 hours. Apoptosis of PBMCs was significantly reduced in a single culture, but it was ineffective in co-culture with HeLa. In co-culture with HeLa, the presence of MSCs significantly increased TGF-β gene expression in PBMCs.

Conclusion: Based on the findings of the present study, the use of MSCs and their condition medium can be considered a therapeutic method in the treatment of cervical cancer, and the use of these cells and their secretions for the treatment depends on several factors, including the number of cells, the duration of culture and the method of collecting medium, and the condition and duration of the treatment, which must be optimized.

Keywords: Adipose tissue mesenchymal stem cells (MSC), Peripheral blood mononuclear cells (PBMC), cervical cancer cell line (HeLa)

The effects of c-Kit Receptor, AKT, and NF-κB signaling pathway inhibitors on the immune evasion mechanisms of multiple myeloma cell line

Abbas Ranjbar[1,2], Parvin Nozari[1], Akbar Hedayatizadeh-Omran[1], Saeid Taghiloo[1], Hossein Asgarian-Omran[1]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2.Gastrointestinal Cancer Research Center, Non-Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran

Background: Multiple myeloma, also known as plasma cell myeloma and simply myeloma, is a relatively rare cancer affecting plasma cells. One of the new emerging hallmarks of cancer cells is to evade the host immune system. Up-regulation of immune checkpoint ligands and secretion of soluble factors are two potential mechanisms leading to the proliferation and survival of cancerous plasma cells survival in the bone marrow milieu. In the current study, the effects of small molecule inhibitors of c-Kit Receptor, AKT, and NF-κB signaling pathways were investigated on the regulation of immune escape mechanisms in multiple myeloma cells.

Methods: The U266B1 human myeloma cell line, as the in vitro model of multiple myeloma, was treated with masitinib as c-Kit Receptor inhibitor, perifosine as AKT inhibitor, and bortezomib as NF-κB inhibitor either in a single or combined format. Cell viability and apoptosis of U266B1 cells were evaluated using MTT and flow cytometry assays, respectively. The relative mRNA expression of programmed death-ligand 1 (PD-L1), poliovirus receptor (PVR) immune checkpoint molecules, as well as IL-6, were determined by Real-Time PCR. Finally, the secretion of IL-6 was measured by ELISA.

Results: Our findings demonstrated decreased proliferation of U266B1 cells after treatment with all applied drugs masitinib, perifosine, and bortezomib. The combined treatment showed more proliferation inhibition effects than the single treatment. Also, a remarkable increase was observed in apoptosis rate after a single treatment with bortezomib, but not masitinib and perifosine. Furthermore, the expression of PD-L1 was decreased after treatment with all drugs which was more remarkable in combined treatment. Regarding PVR, its mRNA expression was decreased after treatment with masitinib and perifosine, but not bortezomib. However, combined treatment of U266B1 cells with bortezomib and perifosine or three inhibitors decreased the expression level of PVR. The mRNA and protein levels of IL-6 were decreased after a single treatment with perifosine and bortezomib as well as in combination form.

Conclusion: Our data showed that c-Kit receptor, AKT, and NF-κB pathway inhibitors not only serve as cytotoxic drugs in multiple myeloma but also interfere with the immune escape mechanisms of malignant plasma cells by disrupting signaling pathways.

Keywords: immune evasion, multiple myeloma, immune checkpoint, small molecule inhibitor, signaling pathways

The impact of killer cell immunoglobulin-like receptor (KIR) genes and human leukocyte antigen (HLA) class one ligand on predisposition or protection against prostate cancer

Mina Roshan Zamir[1], Ali Ariafar[2], Abbas Ghaderi[3], Aliakbar Amirzargar[1]

1. Department of Immunology, Tehran University of medical sciences, Tehran, Iran
2. Urology-oncology Research Center, Shiraz University of medical sciences, Shiraz, Iran
3. Shiraz Institute for cancer research, Shiraz University of medical sciences, Shiraz, Iran

Background: HLA class one ligands and KIRs are key players in the development of Nk cells. We looked into the role of KIR genes, HLA ligands, and KIR-HLA combinations in susceptibility or protection against prostate cancer in the current study.

Methods: Analyze the frequency of 16 KIR genes and 5 HLA ligands PCR-SSP was conducted in 150 prostate cancer patients and 200 healthy controls.

Results: The frequency of KIR2DL5, KIIR2DS5, HLA-B Bw4 Thr80, and HLA Bw4 and T4 gene clusters was lower in prostate cancer patients. Activating KIRs may play a significant role in prostate cancer. in the case of KIR-HLA interactions, the presence of KIR3DL1 in the absence of its ligands favorably correlated with an increase in susceptibility to prostate cancer.

Conclusion: Our data suggest a protective role for activating KIIRs as well as a susceptible role for certain KIR-HLA combinations in prostate cancer.

Keywords: KIR, HLA, KIR-HLA interaction, NK cell

The inhibitory receptors PD1, Tim3, and A2aR are highly expressed during mesoCAR T cell manufacturing in advanced human epithelial ovarian cancer

Behnia Akbari[1], Tahereh Soltantoyeh[2], Zahra Shahosseini[1], Jamshid Hadjati[1], Hamid Reza Mirzaei[1]

1. Tehran University of Medical Sciences, Tehran, Iran.
2. Iran University of Medical Sciences, Tehran, Iran.

Background: The two mainstays of epithelial ovarian cancer (EOC) treatment to date have been chemotherapy and surgery. Recent advancements in cellular immunotherapies, including CAR T cell therapy, have given solid tumors such as EOC hope for a recovery. The effectiveness of CAR T cell therapy may be hampered by extrinsic factors connected to the manufacturing process of CAR T cells and/or intrinsic dysregulation of patient-derived T cells, which may be linked to the cancer itself, cancer stage, and treatment regimen.

Methods: The expression level of the immune inhibitory receptors (i.e., TIM3, PD1, A2aR) in the peripheral T cells of EOC patients and healthy controls was measured during each stage of CAR T cell production in order to investigate the relationship between these factors and CAR T cell exhaustion.

Results: Our findings showed that primary T cells from EOC patients have significantly elevated immune inhibitory receptors expression. Patients undergoing chemotherapy and those with advanced cancer were significantly more likely to have this increase. Moreover, CAR T cell manufacturing process itself can boost the expression of these receptors and further promote CAR T cell exhaustion.

Conclusions: Our findings imply that during the CAR T cell manufacturing process, intrinsic properties of patient-derived T cells and extrinsic factors in CAR T cell production protocols should be taken into account and properly balanced. Additionally, during CAR T cell production, inhibiting signaling by pharmacological or genetic targeting of the immune inhibitory receptors may significantly enhance CAR T cell function and efficacy.

Keywords: TIM3, A2aR, PD1, Exhaustion, CAR T cell manufacturing, epithelial ovarian cancer

The performance of CD137L-Dendritic cells versus patient-derived melanoma cells; Evaluation of induced CTL' function

Maryam Ajami[1], Seyed Mohammad Moazzeni[1]

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Background: Dendritic cell (DC)-based cancer vaccination is a well-known strategy for immunotherapy of cancer. Meanwhile, the efficacy of DC-based immunotherapy relies on critical factors relating to DCs such as the induction method, state of maturation, and proper delivery of antigens. In this study, we attempt to use a different protocol for the development of ex vivo-generated DC-based vaccines against melanoma, which leads to potent antigen-specific immune responses.

Methods: Tissues (melanoma and healthy skin as control) and blood samples were provided from melanoma patients. To induce inflammatory dendritic cells, monocytes isolated from PBMC were cultured in CD137-FC coated 6 well plates for 7 days. For DCs maturation and Ag loading, the cells were treated with melanoma cells extract, STAT3 inhibitor (R848), and IFNγ for the last 18 hours of culture. Induction of specific cytotoxic T cells was done by co-culture of autologous T lymphocytes and tumor Ag pulsed dendritic cells. CTL assay was done by co-culturing of the derived cytotoxic T cells and melanoma cells following apoptosis determination using the AnnexinV/PI kit.

Results: The TCD4+/TCD8+ ratio decreased on day 14 of the co-culturing of DCs and autologous T cells, indicating the induction of cytotoxic T cells. CTL assay showed the highest cytotoxicity at a ratio of 20:1 (CTL: Target). (Target A: autologous melanoma cells, target B: allogeneic melanoma cells, target C: healthy tissue melanocyte).

Conclusion: Indeed, CD137L-DC cells improve the immune response by increasing the capacity of stimulating T lymphocytes, and induction of cytotoxic T cells, probably due to the high expression of adhesion molecules which can extend the signaling time and strengthens the immunological synapse. Therefore, it seems that CD137L-DCs are more efficient APCs for induction of anti-tumor immune response than conventional DCs and seem to be more suitable for immunotherapy of cancers.

Keywords: Melanoma, CD137L-DC, Dendritic cell therapy, Cytotoxic T cell assay, Ex-vivo test.

The relationship between serum IgE level and IL-4 and IL-13 cytokines in colorectal cancer patients

Zahra Mozooni[1], Fatemeh Faraji[1], Sara Sara Minaeian[1], Leyla Bahadorizadeh[1]

1. Institute of Immunology and Infectious Diseases, Antimicrobial Resistance Research Center, Iran University of Medical Sciences, Tehran, Iran

Background: Colorectal cancer (CRC) is the most common malignancy of the digestive system in the world. The immune system is one main constituent of the tumor microenvironment. The discovery of the immune system components that are involved in cancer development has an important role in the detection of novel biomarkers for prognosis, treatment monitoring, and the development of immune-based therapies. This study investigated the serum IgE levels and expression of IL-4 and IL-13 in the tissue and serum of CRC patients and explored their possible association with pathological and clinical factors.

Methods: 36 patients with CRC and 36 healthy individuals were involved in the study. Tissue and blood samples were collected. Serum levels of IgE and IL-4 and IL-13 were analyzed using the ELISA method. The quantitative Real-Time PCR (qRT-PCR) technique was used to assess the expression levels of the cytokines in CRC tissue samples in comparison with the adjacent control tissue.

Results: Our results revealed that the serum level of IL-4 and IL-13 and also their gene expression levels were significantly decreased in CRC patients compared to the controls. Although the serum level of IgE was reduced in patients compared to the control group, it was not significant.

Conclusion: These results indicated that IL-4 and IL-13 levels and serum levels of IgE may serve as potential diagnostic biomarkers for CRC.

Keywords: Colorectal cancer (CRC); IgE; IL-4; IL-13

The role of *Fusobacterium nucleatum* in increasing the aggressive behavior of cancer-associated fibroblasts in colorectal cancer

Fatemeh Eskandari Malayeri[1], Tahmineh Narimani[2], Marzieh Rezeai[1]

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: *Fusobacterium nucleatum*, as a known factor in inducing oncogenic, invasive and inflammatory responses, can lead to an increase in the incidence and progression of colorectal cancer (CRC). Cancer-associated fibroblasts (CAF) are also one of the key components of the tumor microenvironment, which lead to resistance to treatment, metastasis, and disease recurrence with their markers, secretions and functions. This study aimed to investigate the effect of *F. nucleatum* on the invasive phenotype and function of fibroblast cells isolated from normal and cancerous colorectal tissue.

Methods: *F. nucleatum* bacteria were isolated from deep periodontal pockets and confirmed by various tests. CAF cells from tumor tissue and normal fibroblasts (NF) from a distance of 10 cm of tumor tissue were isolated from 5 patients by the explant method and were exposed to secretions and ghosts of *F. nucleatum*. The expression level of two markers, FAP, and αSMA, and the amount of production of two cytokines TGF-β and IL-6 from fibroblast cells were measured by flow cytometry and ELISA test, respectively before and after exposure to different bacterial components.

Results: The expression of the FAP marker was significantly higher in CAF cells compared to NF cells ($p< 0.05$). Also, the expression of IL-6 in CAF cells was higher than that of NF cells. In investigating the effect of bacterial components on the function of fibroblastic cells, after comparing the amount of IL-6 produced between the normal tissue of each patient and his tumoral tissue under 4 treated conditions, it was found that the amount of IL-6 production from the CAF cells of patients in the control group, treated with heated ghosts and treated with paraformaldehyde-fixed ghosts had a significant increase compared to NF cells ($p< 0.05$).

Conclusion: Due to the significant increase in FAP marker expression in fibroblast cells of tumor tissue compared to normal tissue, it seems that FAP can be used as a very good therapeutic marker, especially in patients with high levels of CAF cells.

Keywords: Colorectal cancer, *Fusobacterium nucleatum*, Cancer-associated fibroblasts (CAF), Fibroblast activation protein (FAP), αsmooth muscle actin (αSMA).

The study of the relationship between PD-1 expression in the tumor tissue of patients with colorectal cancer and the clinicopathological characteristics according to tumor sidedness

Zahra Mokhtari[1]

1. Isfahan University of Medical Science, Isfahan, Iran

Background: Activatory or inhibitory immune checkpoints regulate the activity of the immune system. Programmed cell death receptor 1 (PD-1) is an inhibitory immune checkpoint that regulates the immune responses and their overexpression reflects the exhaustion of the immune system. Now, it is a therapeutic target in various types of cancers. Also, its prognostic importance is discussed in various tumors, including colorectal cancer (CRC); however, it is still unclear how it affects the prognosis of CRC. This study aimed to investigate the prognostic value of PD-1 in patients with CRC according to tumor location.

Methods: 136 patients with CRC who underwent curative surgery were enrolled in this study. Immunohistochemical staining was performed for PD-1 and its expression was evaluated in the center of the tumor and invasive margin.

Results: High expression of PD-1 in invasive margin was significantly related to lower T stage (T1/2) in left tumors, low M stage (M0) in right tumors, absence of metastasis in right and left tumors, larger tumor size (≥ 5 cm) in right-sided tumors, absence of recurrence in left-sided tumors (p<0.05). The expression of this marker in any tumor areas was not significantly related to the survival of the patients.

Conclusion: The findings of this study, showed that the high expression of PD-1 is related to the lower stages of the disease, but it is not a good indicator for the prognosis of patients with colorectal cancer.

Keywords: Colorectal cancer, Immune checkpoint proteins, Immunohistochemistry, prognosis, PD1 protein

The survey in the role of LAG3+ tumor-infiltrating lymphocytes in colorectal cancer prognosis; Relationship with sidedness

Marzieh Rezaei[1*], Shirin Tavana[1], Mohammad Hossein Sanei[2], Zahra Mokhtari[1], Zahra Heidari[3], Zahra Sadat Faghih[4]

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Biostatistics and Epidemiology, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran
4. School of Medicine, Shiraz Institute for Cancer Research, Shiraz University of Medical Sciences, Shiraz, Iran

Background: The interplay between tumor-infiltrating lymphocytes (TILs) and tumor cells is a major determining factor in cancer progression. CD45RO seems to be a reliable indicator for predicting prognosis and disease outcome, along with CD3 and CD8 markers. LAG-3 is another important marker that overexpresses on TILs in a variety of cancers and is associated with disease prognosis; however, its prognostic impact is controversial. Hence, in the present study, we aimed to investigate the presence of CD45RO+, LAG3+, CD3+, and CD8+ lymphocytes in CRC tumor tissues and their association with clinicopathological parameters of the disease as well as patients' survival, according to primary tumor locations.

Methods: Expression of CD45RO, LAG3, CD3, and CD8 was Immunohistochemically analyzed in tissue sections from 136 patients with CRC. The percentage of TILs expressing these markers were was separately determined in the invasive margin (IM) and center of the tumor (CT). Their associations with clinical factors and survival were analyzed in the entire cohort, and in the subgroups of patients with right and left/rectum tumors.

Results: Based on our observation, CD45RO+ and CD3+ lymphocytes were the most frequent infiltrated cells in both CT and IM regions of colon tumor tissue. On the other hand, LAG3+ lymphocytes were the least frequent subset in both areas. In univariate analysis, high scores of CD45RO in IM was associated with better overall survival in the entire cohort. High score of CD8 and CD45RO in IM were also associated with improved overall survival in right-sided tumors. However, CD45RO expression correlated with advanced TNM stages (III/IV), in the entire cohort and in right-sided tumors. In the case of LAG3, although the association between the expression of this marker and survival was not found, LAG3+ TILs in CT were associated with higher T-stages in the entire cohort.

Conclusions: Our findings suggest that the clinicopathological and prognostic significance of immune system-related markers such as CD45RO and LAG3 depends on the primary tumor sides. Our results collectively demonstrated that infiltration of CD45RO+ lymphocytes in IM could be an independent prognostic factor in a site-dependent manner.

Keywords: Colorectal cancer, immunohistochemistry, prognostic biomarker, tumor microenvironment

Toxicity assessment of Auraptene in Acute Myeloid leukemia

Majid Ghorbani[1], Fatemeh Ghorbani[2]

1. Department of Hematology and blood banking, Mashhad University of medical sciences, Mashhad, Iran
2. Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran

Background: Acute myeloid leukemia (AML) is a heterogenous and hematopoietic malignancy characterized by aberrant cellular proliferation, an aggressive clinical course, and generally high mortality with poor prognosis. The outcome of chemotherapy depends on the molecular and cytogenetic phenotypes of the distinct AML subtypes. More than 50% of patients will experience relapse due to the large heterogeneity of their AML clones. This study was designed to identify the cytotoxic effects of Auraptene on HL60 and U937 cell lines.

Methods: The cytotoxic effects of Auraptene were measured by Alamar Blue assay (Resazurin) following 24- and 48-hours treatments with different doses of Auraptene. The inducive effects of Auraptene on cellular oxidative stress were investigated by determining cellular ROS levels. The cell cycle progression and cell apoptosis were also evaluated by flowcytometry method.

Results: Our findings revealed that Auraptene decreased AML cellular proliferation by downregulation of Cyclin D1. Auraptene also induces cellular oxidative stress by upregulation of cellular ROS levels. Auraptene induces cell cycle arrest the early and late phases of apoptosis by upregulation of Bax and p53 proteins.

Conclusion: Our data suggest that the anti-cancer function of Auraptene can be mediated by promoting cell cycle arrest and apoptosis and inducing cellular oxidative stress in AML cells. These findings support that Auraptene may be used as a potent anti-cancer agent against hematologic malignancies in the further studies.

Keywords: Auraptene, AML, apoptosis

Validation of miRNAs achieved from GEO database in serum and tissue of breast cancer as a potential diagnostic biomarker

Amirhossein Mardi[1], amin karmani[2], negin karamali[2], Nasibeh Mardafkan[3]

1. Student Research Committee, Tabriz University of Medical Science, Tabriz, Iran, Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
3. Faculty of paramedicine, Tabriz medical university, Tabriz, Iran

Background: Breast cancer, which affects more than 500,000 women yearly, is the most prevalent invasive cancer in women and the leading cause of cancer-related deaths. The current study intends to develop a reliable panel of microRNAs (miRNA) to accurately detect patients with breast cancer in clinical settings.

Methods: Using bioinformatics methods and data achieved from the GEO database, we identify 7 miRNAs which have been elevated in the interstitial fluid of the breast tumor compared with tissue samples. Then, using the Real-time PCR technique, the expression levels of 7 potential miRNAs were examined in the serum samples of 26 breast cancer patients and 15 healthy controls, as well as 14 tumor tissue samples and 14 adjacent normal tissue samples. Then sensitivity and specificity of candidate miRNAs were evaluated via the ROC curve.

Results: GraphPad Prism was used for the data analysis. It demonstrates that detected miRNAs are altered in breast cancer patients' serum when compared to healthy controls, as well as tissue samples.

Conclusion: Based on our findings, serum miRNA measurements from tumors are an essential method for the blood-based identification of breast cancer in Iranian women. We discovered that several miRNAs achieved from GEO database may be potential diagnostic biomarkers with high sensitivity and specificity in breast cancer.

Keywords: breast cancer, bioinformatics, miRNAs

Valproic Acid Inhibits Cell Proliferation and PD-L1-Mediated Tumor Immune Escape through Targeting CIP2A and C-MYC/PI3K/Akt/ mTOR signalling Molecules in Breast Cancer

Elahe Zeinali[1], Vahid Bagheri[2], Esmaeil Rostami[3], Gholamreza Anani Sarab[4]

1. Department of Immunology and Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran
2. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran
3. Department of Laboratory Sciences, Faculty of Para- Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran
4. Cellular & Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

Background: Resistant cells are a critical problem that reduce treatment efficacy of breast cancer. Nowadays, CIP2A and PD-L1 are considered as theraputical challenges in breast cancer, because of responsible for drug resistance and immune evasion respectively. Hence, identifying agents to suppress these factors is great of interest. Specifically, epigenetic drugs can be an effective approach to alter the behavior of genes. Although valproic acid (VPA) as a HDAC inhibitor has certain anticancer properties but molecular mechanism of VPA in breast cancer cells remains to be explored. In this study, we investigated drug effects and molecular mechanisms of VPA, particularly its effect on CIP2A and PD-L1 in breast cancer MCF-7 cell line.

Methods: In this study, MCF-7 cells were treated with various concentration of VPA for 24 h, 48 h, and 72 h. The rate of cell viability was measured by MTT assay. Finally, gene expressions of CIP2A, c-MYC, PI3K, Akt, mTOR and PD-L1 were analyzed by real time PCR and ΔCT method.

Results: VPA showed a growth inhibitory effect in a dose and time-dependent manner in MCF-7 cell line. This effect is achieved by decreasing the expression levels of CIP2A oncogene and its downstream signaling molecules i.e. c-MYC, PI3K, Akt and mTOR. Furthermore, VPA decreased the expression levels of PD-L1 in relation to CIP2A inhibition.

Conclusion: These results indicated that VPA exerts its growth inhibitory effect through targeting the CIP2A and its downstream signaling molecules. In addition to being a CIP2A targeting agent, VPA also inhibits PD-L1 through CIP2A inactivation in MCF-7 cell line. Our findings suggest that VPA can be a novel approach to combat with challenges caused by CIP2A and PD-L1, thereby alone or in combination with existing therapies could be promising strategy to get more efficiencies in treatments for breast cancer patients.

Keywords: Valproic acid (VPA), histone deacetylase inhibitor (HDACi), CIP2A, PD-L1; breast cancer

Cancer Immunotherapy

Cancer combination therapies with Sorafenib and anti-miR-222 in renal cell carcinoma

Leili Aghebati-Maleki[1], Erfan Komijani[2]

1. Immunology research center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Background: The Sorafenib chemotherapy drug is an oral multikinase inhibitor that acts as an inhibitor of cell migration, and viability, induces apoptosis and is considered an effective drug for dealing with renal cell carcinoma (RCC). Recently, the study of combined treatments for the treatment of different types of cancers has received more attention, and this is due to the prevention of drug resistance and better results from these treatments. Therefore, in this study, we investigated the anti-apoptotic and anti-metastatic effects of Sorafenib and anti-miR-222 in RCC cell lines.

Methods: The expression level of PD-L1, Bim, miR-34a, miR-122, miR-513, and miR-570 genes in 786-O and Caki-1 cell lines, before and after treatment with Sorafenib was measured by quantitative real-time polymerase chain reaction (qRT-PCR). Also, changes in cell viability by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test, apoptosis by flow cytometry and cell migration by scratch assay were investigated after treatment with Sorafenib and anti-miR-222 in these two cell lines.

Results: After the treatment of 786-O and Caki-1 cell lines with Sorafenib, an increase in the expression of PD-L1, Bim, miR-34a, miR-122, miR-513, and miR-570 genes was observed. The decrease in cell viability in 786-O and Caki-1 cells that were treated with Sorafenib and anti-miR-222 singly and simultaneously, by examining the results of the MTT test, it was found that this decrease is more significant in the case of combined treatment. Also, the results of flow cytometry showed that the rate of apoptosis increases after the treatment of these two cell lines only with Sorafenib, only with anti-miR-222, and combined treatment with both, a more significant increase was observed after the combined treatment. Reduction of cell migration in 786-O and Caki-1 cell lines treated with Sorafenib and anti-miR-222 alone and in combination after 24 and 48 hours, after examining the results of the scratch assay, it was observed that the prevention of cell migration after combined treatment with Sorafenib and anti-miR-222 It was more significant.

Conclusion: In summary, the results obtained from the present study show that the combined treatment of RCC cell lines with Sorafenib and miR-222 leads to a significant decrease in cell viability and migration, and a significant increase in apoptosis in these cells. These results can be useful for promoting combination therapies in RCC.

Keywords: RCC, Sorafenib, Combination therapy, Apoptosis, Metastasis

Single-cell RNA sequencing revealed the role of cytotoxic CD8+ T cells in response to immunotherapy of targeting PD-1 in head and neck squamous cell carcinoma

Setareh Azarkar[1], Adib Miraki Feriz[1], Hossein Safarpour[2], Arezo Khosrojerdi[2], Fateme Bahraini[3]

1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran.
2. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.
3. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran.

Background: Head and neck squamous cell carcinoma, originating from the mucosal epithelium of the tongue, mouth, nasopharynx, larynx, and throat has a mortality rate of 40-50% per year. Globally, the prevalence of distant metastases in 10-30% of head and neck cancer cases and tumor relapse in 30-50% of patients are substantial clinical challenges. Immunotherapy is rapidly changing the therapeutic landscape in head and neck cancer, increasing survival and lowering toxicity when compared to current treatments. Nivolumab is a human IgG4 antibody that targets PD-1, an inhibitory receptor on activated T cells that inhibits the patient's immune response against cancer cells by blocking the interaction between the PD-1 receptor and its ligands, PD-L1 and PD-L2. Clarifying immunotherapy mechanisms, developing predictive biomarkers, and identifying novel treatment targets all require a deeper understanding of immune cells in the tumor microenvironment Cytotoxic CD8+ lymphocytes are a key component of the TME, as they reflect the host's antitumor immune response. The goal of this study was to evaluate the effect of Nivolumab on subpopulations of Cytotoxic CD8+ cells and their expression of inhibitory ICs, as well as to assess the molecular interactions between cells in head and neck tumor microenvironment.

Methods: To begin, raw single-cell sequencing data from patients with pre-treatment with nivolumab (n = 3) and post-treatment with nivolumab were obtained from the NCBI database using the code GSE195832. Scanpy toolkit was leveraged for data analysis. Initially, data from the quality control phase were removed to remove dead cells, dividing cells, and stressed cells. The Scran package was then used to perform the Normalization step on the data. The batch effect on the samples was then removed with the Combat package. The PCA algorithm was then used to perform dimensional reduction. Finally, cell clustering based on the expression of specific gene markers was performed. In statistical analyses, the Bonferroni formula was used to calculate the Adjusted P-value.

Results: We found that cytotoxic CD8+ cells were increased after treatment with Nivolumab and based on TCGA tumor bulk dataset, it's due with the favorable prognosis. Also, complement activation, classical pathway and humoral immune response mediated by circulating immunoglobulin and complement activation and immunoglobulin mediated immune response and B cell mediated immunity were significantly enriched in cytotoxic CD8+ lymphocytes. Our results indicated that cytotoxic CD8+ lymphocytes had remarkable interactions with exhausted CD8+ lymphocytes in post-Nivolumab therapy. In post-Nivolumab therapy samples, the main signaling axis between exhausted and cytotoxic CD8+ lymphocytes were mediated via the MIF/CD74.

Keywords: Single-Cell RNA-seq, Nivolumab, Immune Checkpoints, Immunotherapy

A systematic review and meta-analysis of immune checkpoint therapy in relapsed or refractory non-Hodgkin lymphoma; a friend or foe?

Zeinab Davoodi-maghadam[1], Farideh Jafari-Raddani[1], Maryam Noori[2], Davood Bashash[1]

1. Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Student Research Committee, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Over the last decades, a paradigm shift has occurred in oncology with the development of immune checkpoint inhibitors (ICIs). Following tremendous successes in solid tumors, interest has risen to explore these inhibitors in hematologic malignancies; while Hodgkin's lymphoma (HL) has shown overwhelming achievements, available data on different types of non-Hodgkin's lymphoma (NHL) vary considerably.

Methods: To the best of our knowledge, no meta-analysis has assessed the efficacy and safety of ICI therapy in relapsed or refractory NHL cases. Meta-analysis of the included studies (n=29) indicated PD-1 may probably be the more attractive ICI target rather than PD-L1 and CTLA-4 in NHL cases. Also, there is a plausible correlation between NHL subtypes and response to ICI therapy.

Results: While MF, ENKTL, RT, and PMBCL showed promising responses to ICI monotherapy, neither FL nor DLBCL had satisfactory responses; further necessitating novel strategies such as the application of ICIs in combination with other lines of therapies. Notably, among different combinations, BTK inhibitors showed an obvious improvement as compared to ICI monotherapy in both FL and DLBCL, however, the best results were obtained when ICI was combined with anti-CD20.

Conclusion: Finally, while most NHL cases who received ICI treatment have experienced mild AEs, larger trials with long-term follow-up are required to confirm the safety, as well as the efficacy, of ICI therapy in NHL patients.

Keywords: immune checkpoint inhibitor, immunotherapy, non-Hodgkin lymphoma, PD-1, PD-L, CTLA-4

Assessing the synergistic impact of inhibiting Prominin1 (CD133) and administering Oxaliplatin for treating colorectal cancer

Zahra Asadzadeh[1], Nima Hemmat[2], Behzad Baradaran[3]

1. Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Colorectal cancer (CRC) is a leading cancer worldwide. The marker CD133, known as a cancer stem cell marker, plays a significant role in drug resistance, migration, and stemness properties of CRC cells. This study aims to investigate the combined effect of CD133 siRNA and Oxaliplatin on the proliferation, migration, apoptosis, and stemness properties of CRC cells in the HT-29 cell line.

Methods: The MTT assay was used to determine the combined effect of CD133 siRNA and Oxaliplatin on the viability of HT-29 cells. QRT-PCR and western blot were used to examine the impact of this combination therapy on CD133 expression at the gene and protein level, respectively. The ability of cell migration was tested by the wound healing assay. Colony and sphere formation were conducted to assess the stemness properties in the combination group. Flow cytometry was performed to investigate apoptosis, cell cycle, and surface expression of CD133 in different groups.

Results: The combination of CD133 siRNA and Oxaliplatin could decrease the IC50 of Oxaliplatin from 32.85 to 19.75 nmol. Besides, silencing CD133 could reduce its expression to about 0.00001 compared to the control group and reduce the protein level to 0.01. CD133 suppression could reduce migration and stemness properties of colorectal cancerous cells. This suppression makes HT-29 cells more sensitive to Oxaliplatin and reduces the effective dose of this chemical drug.

Conclusion: The suppression of CD133 combined with Oxaliplatin treatment could be a promising therapeutic approach for the treatment of colorectal cancer.

Keywords: CD133, Oxaliplatin, Combination therapy, colorectal cancer

Assessment of PSCA Antigen Expression in Prostate Cancer Tissues Using Antibody Conjugated to Iron Nanoparticles (SPIONs)

Tina Nayerpour dizaj[1], Monire Mohsenzadegan[2]

1. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran

Background: Prostate cancer is one of the most widespread cancers in the world. In this study, a new diagnostic method was shown. We used the method of targeted conjugation of anti-prostate stem cell antigen (PSCA) antibody with iron nanoparticles and evaluated the binding properties of this antibody to prostate cancer and benign tissues.

Methods: In this study, we purified and concentrated anti-PSCA antibodies and conjugated them into super magnetic oxide nanoparticles (SPION). Then, iron staining on prostate tissues was performed. At the same time, immunohistochemical staining was also performed on similar tissues to compare the results. In addition, we used benign prostatic hyperplasia (BPH) samples as a control sample.

Results: In adenocarcinoma tissues with iron staining, many blue spots were seen compared to benign tissues, and the number of these spots increases with increasing tumor grade or Gleason score.

Conclusion: Iron staining method optimized by conjugating PSCA antibodies similar to IHC staining shows staining intensity with increasing Gleason score. In addition to sensitivity and specificity, this staining method has higher safety compared with IHC staining.

Keywords: Anti-PSCA antibody, benign prostatic hyperplasia, Prostate cancer, SPION

Catalase loaded Gold Nanoparticles for Hypoxia Alleviation in breast cancer cells

Milad Karimi[1], Nazanin Aghamohamadi[1], Alireza Najafi[1], Reza Falak[1]

1. Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Cancer is the most common disease in the worldwide. Radiotherapy and chemotherapy are now acknowledged as the most efficient treatments for solid tumors in combination with surgical removal. Hypoxia, on the other hand, is brought on by a lack of blood and an inadequate oxygen supply in solid tumors, which reduces the sensitivity of tumor cells and decreases therapeutic benefits. Hypoxia will also hasten the progression of cancer, raise metastasis, and result in resistance to the majority of anticancer medications by upregulation of VEGF, PD-L1, and other related genes. H2O2, as an increased metabolite in the tumor microenvironment causes tumor stability and progression, increases the expression of HIF-1α and increases resistance to tumor radiotherapy. To increase radiotherapy and chemotherapy sensitivity in solid tumors, sufficient O2 delivery and anticancer drug supply are crucial. Research on reducing tumor hypoxia has received a lot of attention recently and has produced promising findings. Catalytic decomposition of H2O2 is proposed as an ideal strategy to increase the concentration of O2 in tumor to overcome hypoxia. Catalase is an enzyme that has been studied for its unique ability to convert H2O2 into molecular oxygen. However, the short half-life of catalase in blood has limited its therapeutic application. So, we used gold nanoparticles to increase half-life of catalase and protect from degradation.

Methods: Herein, we created biocompatible catalase-conjugated gold nanoparticles (Au@cat NPs) that respond to endogenous H2O2 to reoxygenate the TME. BT-549 breast cancer cells were cultured in DMEM/F-12 supplemented with 10% FBS. 24h post seeding, cells replaced with new media and treated in 3 group, Catalase-Gold nanoparticles, gold nanoparticles, and PBS as a control group, and the plates were placed in the jar, after that the jar degassed with the Whitley Jar Gassing System. After 12 hours total RNA extracted and q-PCR was conducted to assessment of target genes and gene expression analysis was done between groups.

Results: Hypoxia-inducible factor (HIF)-1 expression appeared to be reduced and intracellular oxygen levels appeared to be improved after treatment with Au@cat NPs. Gene expression analysis showed that the expression of HIF-1αand subsequent genes, VEGF, and PD-L1 decreased in the Catalase-Gold group compared to control group. In contrast, in the gold group, no significant differences have been observed.

Conclusion: The results of our study showed that Catalase-Gold nanoparticles can reduce hypoxia conditions in the tumor microenvironment by decomposing H2O2 and producing O2. This nanoparticle caused the recovery of hypoxia and reduced the expression of the HIF-1αgene and its subsequent genes.

Keywords: Hypoxia, HIF-1α VEGF, PD-L1, Catalase, Gold

Combination immunotherapy of acute myeloid leukemia by dual PI3K/mTOR inhibitor BEZ235 and TLR-7/8 agonist R848

Saeid Taghiloo[1], Abolghasem Ajami[1], Reza Alizadeh-Navaei[2], Hossein Asgarian-Omran[3]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Gastrointestinal Cancer Research Center, Non-Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran
3. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: Due to the high toxicity of common therapies in AML patients, as well as the development of resistance to treatment, changes in the treatment strategies are required. Thus, the present study was conducted to determine the effect of combinational therapy with BEZ235 (as dual PI3K/mTOR inhibitor) and R848 (as TLR7/8 agonist) on WEHI-3 cell-induced murine leukemia model.

Methods: Sixty BALB/c mice were randomly divided into six groups (n=10). Group I mice with normal diet were considered as normal control. For other five groups, mice were peritoneally inoculated with WEHI-3 leukemia cells to generate leukemic mice. Group II mice received normal diet as leukemic control. Group III, IV, V and VI leukemic mice were treated with cytarabine, BEZ235, R848, and combined BEZ235/R848, respectively. At the end of treatment, five mice from each group were sacrificed, and other mice were monitored for survival analysis. The frequency of T-CD4+, T-CD8+, NK, MDSCs, and exhausted T cells in isolated splenocytes was measured by flow cytometry. T-cell and B-cell proliferation was evaluated via MTT assay. The degranulation and cytotoxicity levels of isolated splenocyte cells in the presence of WEHI-3 cell line (as target of CTL cells) and YAC-1 cell line (as target of NK cells) were also evaluated by flow cytometry and MTT assays, respectively. The status of M1 and M2 macrophages was measured by analyzing the expression of iNOS and arginase-1 via Real-Time PCR. Then, the expression of immune checkpoint ligands including PD-L1, Gal-9 and PVR as well as the expression of cytokines IFN-γ, TNF-α, IL-4, IL-10, IL-12 and IL-17 was investigated by Real-Time PCR.

Results: Our data indicated that the frequency of T-CD4+ cells in leukemic mice was significantly decreased when compared to the normal group. Following combined treatment with BEZ235 and R848, the frequency of these cells was increased. The frequency of MDSCs in leukemic mice showed a significant increasing when compared to the control group; however, the frequency of these cells was decreased after combinational therapy with BEZ235 and R848. Conversely, the frequency of T-CD8+ and NK cells were not significantly difference between study groups. Interestingly, the frequency of exhausted T-CD4+ and T-CD8+ was significantly higher in leukemic mice, whereas it was significantly reduced after combinational therapy. The analysis of iNOS and arginase-1 expression were showed the decreasing of M1 macrophages and increasing of M2 macrophages in leukemic mice, while it was improved by single and combination therapy with BEZ235 and R848. The degranulation and cytotoxicity activities of splenocytes against target cells were significantly increased after combinational treatment. Then, T-cells proliferation were reduced in leukemic mice in compared to control group, which it was improved following single and combinational treatment. The expression levels of PD-L1, Gal-9 and PVR were decreased in leukemic mice after single and combination treatment with BEZ235 and R848. Regarding cytokines evaluation, over-expression of IFN-γ, TNF-α, IL-12 and IL-17 were observed after combinational treatment of leukemic mice with BEZ235 and R848, whereas the expression of IL-4 and IL-10 was down-regulated. Finally, the survival analysis indicated that treatment with BEZ235 and R848 can significantly increase the survival rate.

Conclusion: Taken together, we indicated that the combinational treatment with BEZ235 and R848 could be considered as a potential and powerful treatment strategy in AML patients. However, further clinical studies are required to expand our current findings and to more explore this therapeutic option.

Keywords: Acute myeloid leukemia, WEHI-3, BEZ235, PI3K/mTOR, R848, TLR7/8

Combined blockade of PD-1 and TIGIT is not sufficient to improve the function of CD8+ T-cells in chronic lymphocytic leukemia

Fatemeh Hatefi[1], Hossein Asgarian-Omran[2], Akbar Akbar[1], Hadi Hossein-Nattaj[1], Amir Kahrizi[1], Ramin Shekarriz[3], Ehsan Zaboli[3], Ghasem Janbabaei[8], Mohsen Tehrani[2]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Immunology and Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
3. Gastrointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
4. Department of Hematology and Oncology, Imam Khomeini hospital, Mazandaran university of Medical sciences, Sari, Iran.

Background: Blockade of immune-checkpoint receptors (ICRs) in the treatment of cancers has been mentioned in several studies. Here, we investigated the efficacy of the combined blockade of two ICRs, PD-1 and TIGIT, in restoring functional features of CD8+ T-cells in CLL.

Methods: CD8+ T-cells were separated from the peripheral blood of 11 CLL patients and targeted with malignant B-cells isolated from the same patients. Cells were then stimulated with anti-CD3/CD28 and PMA/ionomycin to assess their proliferative response and cytotoxic activity using MTT and CD107a degranulation assays, respectively. Cytokine production of isolated CD8+ T-cells was also determined using ELISA.

Results: There were no significant differences in the proliferation and cytotoxic activity of CD8+ T-cells co-blocked with anti-PD-1/TIGIT compared to those blocked with anti-PD-1, anti-TIGIT, or the control antibody. There was no significant difference in cytokine production of mentioned groups, either.

Conclusions: The combined blockade of PD-1 and TIGIT failed to restore the proliferation and function of CD8+ T-cells isolated from CLL patients.

Keywords: Chronic lymphocytic leukemia, PD-1, TIGIT, T-cell exhaustion, immune checkpoint blockade

Combining radiotherapy and immunotherapy for enhanced treatment of rectal cancer: a systematic review

Soheil Sadr[1], Elahe Roohbakhsh Amoli Moghadam[1], Hassan Borji[2]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Radio-immunotherapy is a promising treatment that combines radiotherapy with immunotherapy to enhance the efficacy of cancer treatment. This approach aims to use radiation-induced cell death to release tumor-associated antigens, which then activate the immune system to target and kill cancer cells. Furthermore, radiotherapy can induce the expression of immune checkpoint molecules such as programmed death-ligand 1 (PD-L1), which can be targeted by immune checkpoint inhibitors (ICIs). This study aims to investigate previous studies related to radiotherapy and immunotherapy for enhanced treatment of rectal cancer.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on radiotherapy and immunotherapy for enhanced treatment of rectal cancer from January 2000 to January 2023. Fifteen articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Radiotherapy can help enhance the body's immune response to cancer cells by increasing the release of tumor antigens, promoting the infiltration of immune cells, and upregulating the expression of immune checkpoint molecules. Furthermore, radiotherapy can also induce DNA damage and increase the production of pro-inflammatory cytokines, which can further enhance the antitumor immune response. These effects can help make the tumor microenvironment more receptive to immunotherapy and increase the likelihood of a successful treatment outcome. The studies suggest that combining radiotherapy and immunotherapy may be promising for treating rectal cancer.

Conclusion: In conclusion, Radio-immunotherapy is a promising treatment modality for rectal cancer that has the potential to overcome resistance to immunotherapy alone. Preclinical and clinical studies have demonstrated the potential of this approach, although several challenges need to be addressed to optimize its efficacy.

Keywords: Cancer, Colon cancer, Immunotherapy, Radio-immunotherapy

Construction of reprogrammed CAR-T cells for inverting immunosuppressive effects of TGFb1

Shafieeh Mansoori[1], Soheila Ajdary[1], Mohammad Ali Shokrgozar[2], Shahriyar Abdoli[3], Mohsen Basiri[4], Zahra Sharifzadeh[5]

1. Immunology Department, Pasteur Institute of Iran, Tehran, Iran
2. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
3. School of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran
4. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5. Immunology Department, Pasteur Institute of Iran, Tehran, Iran

Background: Chimeric antigen receptor (CAR) T cell therapy has gained remarkable success in relapsed/refractory hematological cancers. However, it has failed in the treatment of solid tumors. TGFb1 is known to be a major suppressive cytokine of T cells in the tumor microenvironment (TME) of solid tumors. CAR-T cells are manufactured by ex vivo engineering of T cells to express a CAR that transmit a stimulatory signal following binding to a surface tumor antigen (STA). In a new platform, it is hypothesized that soluble TGFb1, instead of STA, may convert the inhibitory TGFb signal to a stimulatory one and activate TGFb CAR-T cells. Here, we evaluated the primary functions of a new TGFb-CAR-T cell including its activation and cytokine production in the presence of TGFb1.

Methods: Jurkat T cells were transduced with a CAR construct containing c-myc-tag-TGFbRII-TM-CD28-CD3ζ. 50 x103 transduced cells/well were seeded in a 96-well plate and treated with or without 10 ng/ml TGFb1 for ~26 h. The cells were stained with anti-c-myc-tag and anti-CD69 FITC antibodies to assess CAR expression and CAR-T cell activation by flow cytometry. 48 h after transduction, supernatants were analyzed for IL2 levels using a human ELISA kit.

Results: A significant increase of CD69 expression was observed when TGFbCAR-T cells were cultured with TGFb1 compared to TGFb CAR-T cells without TGFb1 (MFI of 478 Vs. 219), whereas Jurkat cells were markedly inhibited by TGFb1. Furthermore, TGFb CAR-T cells secreted more IL-2 by two fold in presence of TGFb1 rather than untreated TGFb CAR-T cells.

Conclusion: Our results showed that TGFb CAR-T cells can be activated and stimulated in presence of soluble TGFb1. Therefore, TGFb-CAR T cells are suggested as a new platform for the production of T/CAR-T cells resistant to TGFb1.

Keywords: Chimeric Antigen Receptor, Tumor Growth Factor, Immunotherapy, Cancer, Solid Tumors

Dual blockage of both PD-L1 and CD47 enhances the therapeutic effect of oxaliplatin and FOLFOX in CT-26 mice tumor model

Masoumeh Alimohammadi[1], Reza Alimohammadi[2], Haniyeh Ghaffari-Nazari Ghaffari-Nazari[3], Seyed Amir Jalali[3]

1. Tehran University of Medical Sciences, Tehran, Iran.
2. Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Colorectal cancer is a poorly immunogenic. Such property can be reverted by using ICD. However, ICD inducers can also induce the expression of inhibitory checkpoint receptors CD47 and PD-L1 on tumor cells, making CRC tumors resistant to mainly CD8 T cell killing and macrophage-mediated phagocytosis.

Methods: In this study, we examined the therapeutic effect of Oxaliplatin and FOLFOX regimen in combination with blocking antibodies against CD47 and PD-L1. FOLFOX and Oxaliplatin treatment lead to an increase in CD47 and PD-L1 expression on CT-26 cells invitro and invivo.

Results: Combining blocking antibodies against CD47 and PD-L1 with FOLFOX leads to a significant increase in survival and a decrease in tumor size. This triple combining regimen also leads to a significant decrease in Treg and MDSC and a significant increase in CD8 + INF-γ + lymphocytes and M1/M2 macrophage ratio in the tumor microenvironment.

Conclusion: Our study showed triple combining therapy with FOLFOX, CD47 and PD-L1 is an effective treatment regimen in CT-26 mice tumor model and may consider as a potential to translate to the clinic.

Keywords: checkpoint blockade, colorectal cancer, ICD

Effect of Carbon nanocarrier on SW48 cell line

Delaram Aliakbari[1], Moein Sarsangi[1], Zahra Matluobi[2], Amin Kazemi Beydokhti[1]

1. Hakim Sabzevari University, Sabzevar, Iran.
2. Medical University of Sabzevar, Sabzevar, Iran.

Background: Cisplatin (CP) is a cancer drug that is used to treat colon cancer, but it has irreversible side effects. Single-wall carbon nanotubes (SWCNTs) can assist in distributing and stabilizing the cisplatin drug in the body, which can improve cisplatin's effectiveness in treating cancer. The results of this research showed that the use of chemical and polymeric surface modifications with polyethylene glycol (PEG) and chitosan (CS) can better form and load the cisplatin drug into the final carrier, which is promising. Additionally, the use of MTT tests on SW48 showed that the complex can improve cisplatin's effectiveness in treating cancer.

Methods: The carrier (SWCNTs/PEG/CS) was made by special techniques, then the CP drug was loaded on the carrier for 48 hours. SW48 was cultured in DMEM medium with 10% FBS and 100 units/ml of penicillin and 100 µg/ml of streptomycin in a flask. After keeping the cells in an incubator at 37°C and 5% CO2, the plates were made. Cells were exposed to carrier, CP and carrier-CP and after 48 hours the toxicity level was measured by MTT method. In addition, the toxicity test was also performed on lymphocyte cells (normal cells).

Results: The results of 50% inhibitory concentration (IC_50) of SWCNTs-Cisplatin complex modified with CHI and PEG and pure cisplatin on SW48 in 48 hours were 8.04 and 11.01 µg/ml, respectively. However, the prepared nanocarrier did not cause toxicity on the lymphocyte cell line.

Conclusion: The study found that a medicinal complex of single-walled carbon nanotubes with cisplatin, chitosan, and polyethylene glycol can have anti-proliferative effects on different SW48 cell lines. The complex is not toxic to the lymphocyte cell line.

Keywords: Carbon nanotubes, Biopolymers, Cisplatin, Cancer

Efficacy and safety of atezolizumab monotherapy or combined therapy with chemotherapy in patients with metastatic triple-negative breast cancer: A systematic review and meta-analysis of randomized controlled trials

Mina Alimohammadi[1], Fatemeh Faramarzi[2], Alireza Mafi[3]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Mazandaran University of Medical Sciences, Mazandaran, Iran.
3. Isfahan University of Medical Sciences, Isfahan, Iran.

Background: Several success has been recorded with PD-L1 blockade via atezolizumab monotherapy or combination therapy with chemotherapy in patients with metastatic triple-negative breast cancer (mTNBC). Due to the lack of a large-scale study, here we presented a meta-analysis aimed to evaluate the safety and efficacy of this promising strategy in patients with mTNBC. A comprehensive literature search was conducted using electronic databases to identify eligible RCTs.

Methods: Then, twelve studies including 2479 mTBNC patients treated with atezolizumab monotherapy or combined with chemotherapy were included before January 2022. The PRISMA checklist protocol and the I2 statistic were applied for quality assessment and heterogeneity tests of the selected trials, respectively. Fixed and random-effects models were estimated based on the heterogeneity tests, and statistical analysis performed by CMA.

Results: Our pooled findings demonstrated that the median overall survival (OS) and progression-free survival (PFS) were 16.526 and 5.814 months, respectively. Additionally, by comparing efficacy indicators between PD-L1–positive and PD-L1–negative groups, an implicated correspondence between efficacy and the expression of PD-L1 biomarker was detected including OS, PFS, and ORR. Also, Immune-related adverse event incident for alopecia was higher (51.9%) than others across atezolizumab therapy. Moreover, the pooled analysis indicated that the overall rate for lung metastasis (42.8% (95% CI: 29.6-57.2%, I2= 96.910%, p= 0.00, Q= 129.443) was higher compared with bone, brain, and lymph node metastasis.

Conclusion: Atezolizumab showed a manageable safety profile and had a promising and durable anti-tumor efficacy in TMBC patients. Higher PD-L1 expression may be closely correlated to better clinical efficacy.

Keywords: Atezolizumab, mTNBC, efficacy, safety, monotherapy, combined therapy, meta-analysis

Efficacy and safety of pembrolizumab monotherapy or combined therapy in patients with metastatic triple-negative breast cancer: A systematic review and meta-analysis

Mina Alimohammadi[1], Fatemeh Faramarzi[2], Alireza Mafi[3]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Mazandaran University of Medical Sciences, Mazandaran, Iran.
3. Isfahan University of Medical Sciences, Isfahan, Iran.

Background: Metastatic triple-negative breast cancer (mTNBC) is the most invasive subtype with a higher recurrence rate and metastasis. Given the advent of a multitude of research articles on the benefits and risks associated with pembrolizumab, preliminary data from many trials have provided promising results for mTNBC patients. This meta-analysis aimed to combine evidence comparing the available data from several eligible studies and to evaluate the efficacy and safety of pembrolizumab monotherapy or combination therapies for mTNBC.

Methods: A comprehensive literature search was conducted using electronic databases such as PubMed, Web of Science, Scopus, Google Scholar, and Embase up to February 2022 to identify eligible RCTs. The PRISMA checklist protocol and the I2 statistic were applied for the selected trials' quality assessment and heterogeneity tests, respectively. Fixed and random-effects models were estimated based on the heterogeneity tests, and statistical analysis performed by CMA software.

Results: Our pooled findings demonstrated that PD-L1-positive patients had a higher response rate with an ORR of 21.1%. First-line immunotherapy had a better ORR than ≥ second-line immunotherapy. Liver and lung metastasis showed poor response. However, the PD-L1-positive subgroup had no difference rather to the PD-L1-negative subgroup in median OS and PFS following pembrolizumab therapy. The pooled incidence of immune-related adverse events was 22.7% during pembrolizumab therapy. The patients with mTNBC had a moderate response to pembrolizumab therapy.

Conclusion: PD-L1-positive, first-line immunotherapy, combination therapy, and non-liver/lung metastasis could predict better response to pembrolizumab treatment. Patients with PD-L1-positive tumors could be more survival benefits from immune checkpoint therapy with chemotherapy.

Keywords: pembrolizumab, mTNBC, efficacy, safety, monotherapy, combined therapy, meta-analysis

Evaluation of anti-cancer effect of a tandem diabody against PD-1 and CTLA-4 on breast cancer cells

Nafise moosavi[1]

1. Isfahan University of Medical Sciences, Isfahan, Iran.

Background: Many studies showed that co-targeting of immune checkpoints can improve the efficacy of cancer treatment. Different types of bispecific antibodies have been produced for cancer treatmen. One of the common types of bispecific antibodies is single chain antibodies such as diabody. Here, we aimed to evaluate the anticancer effect of a bispecific diabody against two immune checkpoints, PD-1 and CTLA-4.

Methods: Cytotoxicity of anti-PD-1/anti-CTLA-4 diabody against MCF-7 and MDA-MB-231 cells was evaluated using MTT method. Apoptotic effect of the diabody was detected using Annexin/propidium iodide method. The apoptosis induction was also checked by western blotting. The effect of protein on cell cycle was examined using flow cytometry.

Results: Cytotoxicity of the diabody against MDA-MB-231 cells was more than MCF-7. Cell survival at 400 nM concentration of the diabody was 42% for MDA-MB-231 and 69% for MCF-7. The result of the statistical test shows that the lethal effect of diabody and doxorubicin in these concentration, 25-50-100-200 nM and 21.8, 43.7, 81.5, 175 nM in MCF-7 with MDA-MB-231 with P-value less than 0.05 is significant .The diabody at concentration of 400 nM led to 31.7% apoptosis according to Annexin/propidium iodide method. The western blot analysis showed that the diabody at concentration of 400nM increased the ratio of Bax/Bcl-2 form 0.56 to 0.64. Cell cycle evaluation showed that the diabody led to cell cycle interruption, leading to cell cycle arrest in G2 and decrease S.

Conclusion: Our results indicate that the toxicity of this diabody was more in MDA-MB-231 (PD-L-1 overexpressing cell line) then MCF-7 (PD-L-1 low expressing cell line). Immune checkpoint inhibitors can improve breast cancer treatment particularly PD-L-1 overexpressing type. This diabody could be a potential anticancer agent and should be evaluated for more invitro and invivo experiments Summary of the overall findings and the importance of the study.

Keywords: Cancer, diabody, Immmune checkpoint, Cytotoxicity

Evaluation of the effect of specific diabody antibody against CTLA-4 and PD-1 on survival and migration of breast cancer cells

Kosar Amini[1], Vajihe Akbari[1], Nafiseh Esmaeil[1], maryam Abbaspour[1]

1. Medicine University of Isfahan, Isfahan, Iran.

Background: Dual-specificity antibodies such as diabodies have become an emerging therapeutic approach in cancer treatment due to their ability to simultaneously target two different antigens. This approach can provide a more efficient and effective treatment option for patients with cancer, as they can target multiple pathways simultaneously. Studies have shown that diabodies targeting CTLA-4 and PD-1 can lead to increased antitumor activity both in vitro and in vivo. However, further research is needed to fully investigate the effectiveness and safety of this approach, and to determine the optimal dosing and timing of treatment. In this study, the function of diabody expressed on the ability to survive, wound healing, and migration was investigated.

Methods: After the expression and purification of diabody, the effect of diabody on breast cancer cells MDA-MB-231 and MCF-7 was investigated. The direct effect of diabody on the survival ability of cancer cells in the presence of peripheral blood mononuclear cells (PBMCs) was investigated by the MTT method. The effect of diabody on the proliferation of CFSE-labeled cancer cells in the presence of PBMC was determined by flow cytometry. Also, the effect of diabody on the migration ability of cancer cells was investigated by the transwell migration assay and the wound healing ability by the scratch test method.

Results: In the MTT test, Diabadi was able to reduce the viability of cancer cells, especially MDA-MB-231. Diabadi was able to inhibit cell migration in these two lines, and this inhibitory effect on MDA-MB-231 cells decreased more clearly (42%). Diabody also inhibited the ability of wound healing in cancer cells.

Conclusion: The diabody developed in this study showed promising results in inhibiting the proliferation, migration, and metastasis of breast cancer cells. However further studies are needed to investigate the safety and efficacy of this diabody in clinical trials.

Keywords: Diabadi, Breast cancer, CTLA-4 and PD-1

Generation and characterization of PDL1-CAR NK cells as a therapeutic approach for cancer immunotherapy

Mahsa Yazdanpanah-Samani[1], Susanne Michen[2], Amin Ramezani[3], Zohreh Mostafavipour[4], Achim Temme[2], Nasrollah Erfani[5]

1. Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences
2. Section Experimental Neurosurgery/ Tumor Immunology, Medical Faculty Carl Gustav, TU Dresden
3. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences
4. Department of Biochemistry, School of Medicine, Shiraz University of Medical Sciences
5. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences

Background: Immune cell engineering techniques termed chimeric antigen receptor (CAR) have emerged as a cutting-edge approach in cancer immunotherapy. Natural killer (NK) cells are the highly toxic immune effector cells that can be a superior alternative to T cells to generate an allogeneic off-the-shelf product, due to the lack of need for human leukocyte antigen (HLA) matching and the lack of potential for graft versus host disease (GvHD). Programmed death-ligand 1(PD-L1) is a key inhibitory molecule in suppressing the immune response in the tumor microenvironment. Generation of CAR-engineered cells against PD-L1 blocks the inhibitory signal to immune cells, changing the tumor microenvironment in favor of immune system. This study aimed to design and generate PDL1 CAR-NK cells.

Methods: Sequences encoding leader, anti-PDL1 single-chain, hinge, transmembrane domain, signaling domains of CD28 and CD3zeta, and IL-15 were obtained from data banks. The final sequences synthesized. PCR, restriction pattern, and sequencing methods used to confirm the construct. HEK293T cells used to make 3rd generation lentiviral particles. 3 different vector ratios used to set up the experiment. HEK293T cells transduced and based on puromycin kill curve, antibiotic treatment performed. PDL1-CAR expression and binding to target molecule was evaluated by flow cytometry. Primary NK cells isolated by autoMACS-Pro device, then activated and expanded, and transduced with MOIs 1-5. Growing curve after transduction, PDL1 CAR expression and binding ability was evaluated. IL-15 expression was determined by ELISA.

Results: PCR, enzymatic restriction pattern, and sequencing confirmed the PDL1-CAR construct. Stable HEK293T PDL1-CAR cell line generated by puromycin treatment. More than 99% of isolated HEK293T-PDL1-CAR cells expressed the CAR molecule. Activated NK cells were transduced. Transduction efficiency was less than 10% for MOI 1 and more than 40% for MOI 5. The growing curves showed decreasing trend from day 5 to 9. IL15 secretion for PDL1-CAR-HEK293T and PDL1-CAR-NK cells in best conditions showed 551 pg/mL and 59 pg/mL, respectively.

Conclusion: NK cells transduced and expressed PDL1-CAR, successfully. IL-15 secretion could help expansion and persistence of NK cells. Still, there is more space to improve this approach.

Keywords: Adoptive cell therapy, Natural killer cells, CAR-NK, PD-L1

Hypoxia induction by the Whitley Jar Gassing System

Milad Karimi[1], Nazanin Aghamohammadi[1], Reza Falak[1]

1. Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Hypoxia is a typical characteristic of solid tumors and plays a key role in cancer progression. Hypoxia promotes cancer growth, invasion, and metastasis, as well as resistance to chemotherapy and radiation. Hypoxia-inducible factor-1 alpha (HIF-1α) is a major transcription factor involved in the hypoxic response of cancer cells, activating hundreds of genes such as VEGF that play an important role in angiogenesis, proliferation, invasion, and metastasis. Oxygen tensions may be adjusted in vitro, and hypoxia can be produced using several ways and will be applicable in invitro research. Using of hypoxia incubators or chambers filled with a gas mixture containing a predetermined quantity of oxygen, and HIF-1αchemical stabilization by CoCl2 is the easiest way to inducing hypoxia. Due to the limitations of hypoxic incubator availability, we used the Whitley Jar Gassing System to induce hypoxia in BT549 cancer cell line. The Whitley Jar Gassing System is the simplest, fastest, and most cost-effective way to set up anaerobic or microaerobic gas jar conditions.

Methods: In this study BT-549 breast cancer cells were cultured for the hypoxia induction, 24h post-seeding, cells were replaced with new media, and the plates were placed in the jar, after that the jar was degassed with the Whitley Jar Gassing system. 6- and 12-h time periods were selected for hypoxia induction. Simultaneously, control group, the cells were kept in a humidified incubator with 21% O2 (normoxia). At the end of time points total RNA was extracted and the q-PCR was done for assessment of related gene expression and analysis was done between groups.

Results: The results showed that there is a significant increase in the expression of VEGF in 6h in the Hypoxia group compared to the control group but no significant increase observed in HIF-1α gene expression. In contrast, in 12 hours' time period, significant increases in the expression of HIF-1αand VEGF observed in the Hypoxia group compared to the control group.

Conclusion: Therefore, it seems that the Whitley Jar Gassing System can be used for hypoxia induction in vitro, although it is necessary to confirm results by using other techniques like western blotting.

Keywords: Hypoxia, HIF-1α, VEGF, Whitley Jar Gassing System

IL-25 impact on malignant B cells survival and T cells activation in chronic lymphocytic leukemia

Mehrnoosh Pashaei[1], Farahnaz Ghahremanfard[2], Fatemeh Pak[2], Parviz Kokhaei[3]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran
3. Department of Immunology, Arak University of Medical Sciences, Arak, Iran

Background: T cell dysregulation and shift to T helper 2 responses, boosting tumor microenvironment support, contributes to survival of leukemic B cells in Chronic Lymphocytic Leukemia (CLL). Interleukin (IL)-25 is involved in the initiation of Th2 cell responses. Signal transduction of IL-25 begins with the heterodimer receptor IL-17RA/IL-17RB. The presence of IL-25 in the tumor microenvironment may affect the supportive effects of T cells in the surrounding tumor cell environment. The purpose of this study was to evaluate the role of IL-25 in biology of CLL.

Methods: IL-17RB expression in CD3+ and CD19+ cells was assessed in isolated peripheral blood mononuclear cells (PBMCs) of CLL patients and healthy subjects by real-time PCR and flow-cytometry. B cells were positively enriched from PBMCs using immunomagnetic beads (MACS). PBMCs and purified leukemic B cells were cultured with recombinant human IL-25 (20ng/ml) for 72h, then the viability and apoptosis of cultured cells were measured by MTT assay and AnnexinV/7AAD. Furthermore, the levels of CD69 expression on T lymphocytes and IL-17RB in T and B cells were determined by flow cytometry.

Results: The basal level of IL-17RB expression in CLL patients was significantly higher than that in control individuals. In addition, the percentage of IL-17RB+/CD3+ and IL-17RB+/CD19+ cells as well as CD69+/CD3+ cells increased after 72h of culture with IL-25 in CLL patients compared to healthy subjects. IL-25 also reduces the apoptosis rate of tumor cells.

Conclusion: IL-25 activates T cells in CLL patients and reduces B cell apoptosis. Therefore, IL-25 might play a role in increasing tumor cell viability by expressing inflammatory receptors, such as IL-17RB, and may play a role in the pathogenesis of CLL.

Keywords: Apoptosis, Chronic lymphocytic leukemia, CD69, Interleukin-25, IL-17RB

Immunotherapy of Malignant Melanoma: a case report

Rohollah Masumi[1], Fatemeh Nasiri-Dashtaki[2], Leila Nasirinikoo[3]

1. Cancer Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
2. DVM, Student of Veterinary Medicine, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
3. Oncology Ward Nurse, Ayatollah Kashani Hospital, Shahrekord, Iran

Background: Melanoma is a type of skin cancer (uncontrolled growth of melanocytes) of unknown cause. It is less common than other skin cancers but has a much higher risk because if it is not diagnosed and treated in time, it can spread throughout the body.

Patient Report: A 70-year-old woman came in late 2019 with a complaint of a change in the size of a mole in the heel. No other symptoms such as pain, itching, or discoloration were observed. To make a final diagnosis, a sample was taken and a CT scan was performed to check the spread and metastasis. After the surgery, to prevent the progression of the disease, interferon ampoules were injected subcutaneously for 6 months every other day, but due to side effects such as itching, shedding, and hair loss, the drug was discontinued. Ultrasound of the groin area was performed to check the presence of mass and metastasis at a certain time interval. After 6 months of surgery, a lump the size of a walnut was observed under the knee without color change. The metastasis was confirmed by ultrasound and other diagnostic methods. This mass was also surgically removed. By performing an ultrasound and check-up every two months, disease progress and liver involvement were diagnosed. To prevent disease progression and treatment, nivolumab (3 mg/kg) was prescribed as an intravenous infusion every 3 weeks. Nivolumab is an anti-PD1 drug that activates T cells against cancer cells and destroys them. This is called immunotherapy.

Conclusion: After using nivolumab, during the check-up and ultrasound, it was reported that the size of the liver mass remained stable and reduced. Currently, this treatment continues and the patient has responded well to the treatment.

Keywords: Cancer Immunotherapy, Anti-PD1, Malignant Melanoma, Immune System

Immunotherapy with stem cells on breast and colon cancer; Systematic Review

Soroush Partovi Moghaddam[1], Ashkan Hajjafari[1], Soheil Sadr[2], Hassan Borji[3]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Science and Research Branch, Tehran, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: In recent years, immunotherapy for cancer therapy has made significant progress, particularly in stem cell immunotherapy. The goal of stem cell immunotherapy is to prevent the onset of cancer by using stem cells that self-renew and differentiate into different types of cells, including immune cells. To investigate the most recent clinical findings on the use of immunotherapy effects of stem cells in colon and breast cancer treatment, a systematic review of published articles was conducted.

Methods: The study searched nine databases, including PubMed, Scopus, and Google Scholar, for relevant articles published between January 2000 and January 2023. From the search results, 20 articles with complete abstracts were included in the study, and data analysis was performed using R version 4.2.1 artificial intelligence software.

Results: Based on the study results, stem cell immunotherapy demonstrated to be highly effective in cancer treatment research. By targeting drug delivery to cancer cells, stem cells can minimize toxicity to healthy cells while targeting drug delivery to cancer cells. Furthermore, stem cells release TRAIL, a pro-apoptotic cytokine that induces apoptosis in colon and breast cancer cells. The secretion of anti-tumor factors by stem cells, such as Interleukin 12 (IL-12) and IL-24, also inhibits tumor growth and angiogenesis. The use of bone marrow mesenchymal stem cells has also been shown to be highly effective in the treatment of colon and breast cancer. Additionally, adipocyte mesenchymal stem cells showed great promise for treating breast cancer.

Conclusion: In conclusion, immunotherapy leveraging stem cells to target and destroy cancer cells has shown promise as a cancer treatment. Using stem cells to deliver targeted drugs to cancer cells while minimizing toxic effects on healthy cells is an effective way to target drug delivery to cancer cells.Keywords: Immunotherapy, Stem cells, Cancer treatment, Mesenchymal stem cells

Keywords: Immunotherapy, Stem cells, Cancer treatment, Mesenchymal stem cells

Investigate the effects of silver and zinc nanoparticles synthesized from Falcaria vulgaris on acute myeloid leukemia

Maryam Almasi[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[2], Naser Abbasi[2], Navid Etemadi[3], Ali Pirnezhad[4]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.
2. Biotechnology and medicinal plants research center, Ilam University of medical sciences, Ilam, Iran.
3. DVM Graduated, Faculty of Veterinary Medicine, Urmia University, and Urmia, Iran.
4. Central Laboratory, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Background: The desire to prepare and use nanoparticles in the pharmaceutical industry is increasing, but nanoparticles resulting from chemical methods cause environmental damage. Green production of nanoparticles is a nature-friendly method and has priority over chemical methods. In this study, we investigated the ability of Falcaria vulgaris aqueous extract grown in vitro conditions for the biosynthesis of zinc and silver nanoparticles (ZnNPs and AgNPs).

Methods: In vivo design, induction of acute myeloid leukemia was done by 7, 12-Dimethylbenz[a]anthracene (DMBA) in 80 mice. Then, the animals were randomly divided into 8 groups: AgNO3, Zn (NO3)2, F.vulgaris, AgNPs, ZnNPs, daunorubicin, untreated, and control. These nanoparticles were characterized by fourier-transform infrared spectroscopy (FT-IR) spectroscopy, ultraviolet–visible spectroscopy (UV–Vis.), X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), and transmission electron microscopy (TEM) analysis.

Results: By analyzing real-time polymerase chain reaction, sphingosine-1-phosphate receptor-1 and sphingosine-1-phosphate receptor-5 mRNA expression in lymphocytes were significantly ($p \leq 0.05$) raised by treating the leukemic mice with ZnNPs and AgNPs and daunorubicin. ZnNPs and AgNPs similar to daunorubicin, significantly ($p \leq 0.05$) reduced the pro-inflammatory cytokines (IL1, IL6, IL12, IL18, IFNY, and TNFα), and the total white blood cell (WBC), blast, monocyte, neutrophil, eosinophil, and basophil counts and enhanced the anti-inflammatory cytokines (IL4, IL5, IL10, IL13, and IFNα) and the platelet, lymphocyte, and red blood cell (RBC) parameters as compared to the untreated mice.

Conclusion: The results of the chemical description confirm that the synthesized nanoparticles of, F.vulgaris have anti-acute myeloid leukemia effects by increasing the significant amount of anti-inflammatory cytokines.

Keywords: acute myeloid leukemia, Ag nanoparticles, daunorubicin, Falcaria vulgaris, zink nanoparticles

Investigating the effects of mesenchymal stem cells carrying Coxsackie oncolytic virus in the treatment of colorectal cancer in mice model

Reza Karbalaee[1], Morteza Izadi[2], Bahman Jalali Kondori[3], Ruhollah Dorostkar[4], Seyed Morteza Hosseini[5], Hadi Esmaeili Gouvarchinghaleh[6]

1. Students research committee, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3. Baqiyatallah Research Center for Gastroenterology and Liver Diseases (BRCGL), Baqiyatallah University of Medical Sciences, Tehran, Iran
4. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
5. Medicine, Quran, and Hadith Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
6. Assistant Professor in Immunology, Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: Colorectal cancer is the third most common cancer in the world and its mortality rate is approximately 50% of patients. Despite the advances in treatment methods, in most tumors, tumor removal during surgery is considered to be the only effective treatment method. However, about 15-95% of patients show recurrence after surgery. Chemotherapy, is one of the most common treatments for this disease, despite the remarkable success it has had in treating all types of cancers; it always faces two basic problems including drug resistance of cancer cells and side effects on healthy cells. For this reason, nowadays, much attention has been directed toward the use of multifactorial combined treatments. This study aims to investigate the effects of mesenchymal stem cells carrying the Coxsackie oncolytic virus in the treatment of colorectal cancer in mice models.

Methods: After culturing CT-26 cells (colorectal carcinoma cell line), colorectal cancer mouse modeling was done by injecting 5×106 cells into the left flank of female BALB/c mice. After observing the palpable tumor, they were treated with mesenchymal stem cells carrying Coxsackie oncolytic virus (105 cells twice with an interval of one week-intratumoral). 10 days after the last treatment, half of the mice in each group to check the effectiveness of the mentioned treatments, they were sacrificed. The other half of the mice in each group were used to check the lifespan of the mice in response to the treatment. A statistical difference of less than 0.05 was considered a significant level.

Results: The results of the present study showed that the mice receiving the treatment had significantly more favorable survival curves and slower tumor growth rates than the tumor-bearing mice that received only single-agent treatment and/or negative control mice. They did, they have. It also significantly increased the production of nitric oxide and lactate dehydrogenase in the culture of spleen cells of mice with tumors. In addition, it significantly increased the secretion of IFN-γ and conversely decreased the secretion of IL-4 and TGF-β in the spleen cell population compared to the control group.

Conclusion: According to the obtained results, it seems that the use of mesenchymal stem cells carrying the Coxsackie virus increases the effectiveness of anti-cancer treatment compared to the oncolytic virus alone.

Keywords: Colorectal cancer, Mesenchymal stem cell, Coxsackie oncolytic virus

New insights into the role of bispecific antibodies in colorectal cancer therapy

Omid Vakili[1], Farideh Ghalamfarsa[2], Seyyed Hossein Khatami[3], Ahmad Movahedpour[4], Ghasem Ghalamfarsa[5]

1. Department of Clinical Biochemistry, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Medical Biotechnology, School of Advanced Medical Sciences & Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Clinical Biochemistry, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Behbahan Faculty of Medical Sciences, Behbahan, Iran
5. Department of Microbiology & Immunology, School of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran

Background: Colorectal cancer (CRC) is defined as a life-threatening gastrointestinal malignancy. CRC therapy is still a global health concern; however, developing bispecific antibodies (BsAbs) is currently considered to be a promising approach for cancer therapy.

Methods: In this study, the MEDLINE®/PubMed database, as well as the Embase/Scopus were searched to achieve articles published in the relevant field. In this context, “colorectal neoplasm” or “colorectal cancer”, “antibodies, bispecific”, “antibodies, monoclonal”, and “immunotherapy” were principally used as MeSH keywords. Furthermore, an advanced search strategy was employed using ((“colorectal neoplasm” OR “colorectal cancer”)) AND “antibodies, bispecific” AND “immunotherapy”. For a more efficient search performance, inclusion and exclusion criteria were considered as follows: All related controlled clinical trials, in vitro evaluations, and in vivo studies were included, while Meta-analyses were excluded.

Results: BsAbs, as a large family of molecules designed to realize two distinct epitopes or antigens, can be beneficial micro-gadgets to target the tumor-associated antigen pairs. In the case of CRC, solitomab (BiTE type), catumaxomab (Triomab type), MEDI565 (BiTE type), MGD007 (DART type), duligotuzumab (DAF type), vanucizumab (CrossMAb type), and TF2 (DNL type) are considered the major bispecific antibodies in CRC therapy. The corresponding bispecific antibodies respectively target CD3/EpCAM, CD3/EpCAM, CD3/CEA, CD3/gpA33, EGFR/HER3, Ang2/VEGFA, and CEA/HSG antigens. Among all the aforementioned antibodies, catumaxomab is the only FDA-approved medication, and the others are still under phase I or II clinical trials.

Conclusion: The success of clinical trials on blinatumomab demonstrated the potential of BsAbs to achieve the goal of cancer therapy. Although the BsAbs currently appear to be undesirable agents, several approaches (incl. developing tumor Ag selection, improving the delivery processes, and using potential synergistic strategies) are being investigated to increase their activity and limit their toxicity, and thus bispecific antibodies are converting into emerging therapeutics to combat CRC, as well as many other cancers.

Keywords: Colorectal neoplasm, Bispecific antibodies, Immunotherapy, Neoplasm antigens

Potential targets for cancer immunotherapy in natural killer cells' immune checkpoint molecules: a systematic review

Pouria Ahmadi Simab[1], Soheil Sadr[2], Neda Shahvali[3], Hassan Borji[2]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
2. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Pathobiology, Faculty of Veterinary Medicine, Shushtar Branch, Islamic Azad University, Shushtar, Iran

Background: Natural killer (NK) cells may be used in immunotherapy to treat various cancers, according to recent studies. They play crucial roles in tumor surveillance and control. As a result, the functions of immunological checkpoint molecules and receptors in the control of NK cell function as well as their potential use in tumor immunotherapy is the main objective of this systematic review.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on Potential targets for cancer immunotherapy in natural killer cells' immune checkpoint molecules from January 2016 to December 2022. Twenty-five affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Human NK cells are crucial and play a vital role in the development of cancer therapeutics. In the presence of the tumor microenvironment, NK cells transform into dysfunctional cells with increased expression of inhibitory immune checkpoints, such as the non-HLA-class I-specific inhibitory receptors PD-1, TIGIT, CD112R, CD96, IL-1R8, and TIM-3, and decreased expression of activating receptors (such as NKG2D and CD226). A novel inhibitory immunological checkpoint route in NK cells is adenosine-A2AR signaling. The anticancer activity of NK cells can be restored by blocking these inhibitory checkpoint molecules with immune receptor inhibitors and activating co-stimulatory receptor signaling by boosting co-stimulatory receptor expression.

Conclusion: Many primary studies have been conducted and are still being conducted at various stages of the clinical process to support the efficacy of NK cell-targeted immunotherapies based on immune checkpoint molecules in NK cells. The safety, tolerability, and therapeutic effectiveness of medications that target these checkpoints as well as their combination therapy require additional study.

Keywords: Cancer immunotherapy, Natural killer cells, Immune checkpoint molecules, Antigen

Small cells to solve a big problem (cell therapy for the treatment of blood malignancies)

Setayesh Jabbari[1], Mina Ghorbani Mazar[1]

1. Department of Medical Laboratory Sciences, Varastegan Institute for Medical Sciences, Mashhad, Iran

Background: In most blood malignancies, the uncontrolled growth of cells causes the disease. Cell therapy means culturing living cells that repair damaged tissue after entering the body. Because radiation and chemotherapy are associated with recurrence, the development of other cancers, and other challenges, scientists are looking for new ways to treat cancer.

Methods: Related articles were studied from various databases such as Science Direct, Google Scholar, and PubMed. About 60 articles were selected and we focused on articles published during 2019-2022. Data were collected and analyzed based on the findings.

Results: Among the cells used in cell therapy, the following can be mentioned:1- Stem cells: These cells can directly destroy tumor cells (an effect called graft-versus-tumor after allogeneic transplantation) and help to produce blood cells after radiation therapy and chemotherapy.2- Chimeric antigen receptor (CAR) T cells: The variable chain of immunoglobulin that is against cancer cells binds to the constant part of the T cell receptor and targets the cells that express that antigen.3- Natural killer (NK) cells: These cells can destroy tumor cells through cytotoxicity, they can also combine with chimeric antigens and attract other immune cells in addition to their activity. One of the most important features of NK-CARs is off-the-shelf immunotherapy, which is always available and ready.4- Invariant natural killer (Ink) T cell: Activates innate immune cells by expressing the CD40L gene, and they play an important role in killing tumor-associated macrophages. These cells enhance anti-tumor immune responses by activating anti-tumor cells.

Conclusion: The best outcome for accepted cell therapy is a safe, off-the-shelf, universally available product that can be easily produced in sufficient quantities. Cell therapy faces many challenges, such as removing cells in the tumor microenvironment, being time-consuming, loss of tumor antigens, etc.

Keywords: Therapy cell, Car-T cell, Ink-T cell, NKC

Small molecule cabozantinib improves response to anti-HER2 monoclonal antibody therapy via attenuation of MDSC recruitment and function

Vahid Khaki Bakhtiarvand[1], Khadijeh Ramezani-Ali Akbari[1], Seyed Amir Jalali[1], Mohammad Hojjat-Farsangi[2], Mahmood Jeddi-Tehrani[3], Fazel Shokri[4], Mahdi Shabani[1]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Oncology-Pathology, BioClinicum Karolinska University Hospital Solna and Karolinska Institute, 17164 Stockholm, Sweden
3. Monoclonal Antibody Research Centre, Avicenna Research Institute (ACECR), Tehran, Iran
4. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: In breast cancer, immature myeloid cells named myeloid-derived suppressor cells (MDSCs) are the major immunosuppressive cell type impairing anti-tumor responses in the TME. Considering these activities, targeting MDSCs seems to improve the efficacy of anti-HER2 antibody-based immunotherapy in this malignancy.

Methods: BALB/c mice were inoculated with 4T1 and 4T1-HER2 murine tumor cell lines and after 7 days, the mice were divided into different groups. Cabozantinib was orally administrated for 15 consecutive days, and anti-HER2 monoclonal antibody (mAb) 1T0 was intraperitoneally injected twice a week. Tumor size was measured every other day.

Results: Our findings indicated Cabozantinib combined with anti-HER2 mAb dramatically reduced tumor growth and increased tumor rejection (p=0.0001). Flow cytometry analysis showed MDSC population decreased in TME, lymph nodes, and spleens by roughly 20%, 0.8%, and 35%, respectively. MDSC suppressive phenotype was altered through inhibition of the expression of immunosuppressive factor Arg-1. Cytokine profiling of different groups indicated that the level of INF-γ was approximately two times higher than that in the control group, and IL-17 increased compared to the control group. However, IL-4 level was significantly reduced in the groups treated with Cabozantinib. These could bring about a 10% increase in CD8+ infiltration into the tumor bed and activation of tumor-draining lymph nodes and splenic T-lymphocytes. Here, we illustrated how Cabozantinib enhanced the efficacy of anti-HER2 mAb immunotherapy in a 4T1-HER2 murine breast cancer model.

Conclusion: Here, we illustrated how Cabozantinib enhanced the efficacy of anti-HER2 mAb immunotherapy in a 4T1-HER2 murine breast cancer model. Cabozantinib treatment immunologically changed the TME by reducing suppressive myeloid cells and inducing neutrophil-like cells. Collectively, our data provide preclinical evidence for using Cabozantinib to reshape the primary TME, enhancing the effectiveness of anti-HER2 mAb immunotherapy in primary breast cancer.

Keywords: 4T1-HER2, Breast cancer, Cabozantinib, Immunotherapy, MDSC, Monoclonal antibody

Soluble and Immobilized Anti-CD3/28 Distinctively Expand and Differentiate Primary Human T Cells: An Implication for Adoptive T cell Therapy

Tahereh Soltantoyeh[1], Behnia Akbari[1], Hamid Reza Mirzaei[1], Jamshid Hadjati[1]

1. Tehran University of Medical Sciences, Tehran, Iran

Background: Immune cell-based cancer therapies have led to a paradigm shift in the treatment of patients with cancers. Nowadays, a vast majority of cancer immunotherapies have used genetically modified T cells to target tumors. Stimulation and ex vivo expansion of T cells has always been a critical part of adoptive T-cell therapy (ACT). Through transducing signals, one, two, and three, anti-CD3 and anti-CD28 monoclonal antibodies (mAbs) along with interleukin-2 (IL-2), are essential for in vitro T cell activation. Terminal differentiation and replicative senescence are the main barriers of the ACTs during the manufacturing of engineered T cells ex vivo.

Methods: In this study, we aimed to compare the T cell activation protocol that we developed in our lab (soluble anti-CD3/28 mAbs) with a common T cell activation protocol (immobilized anti-CD3/soluble anti-CD28) in terms of T cell expansion, activation, immunophenotype, and cellular fate.

Results: We observed that T cells were equally expanded in both protocols. Notably, our modified protocol promoted the outgrowth of CD8+ T cells post-activation. Concerning the low concentrations of both soluble anti-CD3 and anti-CD28, the modified protocol could significantly enrich memory T cell subsets.

Conclusion: In conclusion, our data demonstrated that the soluble CD3/28 mAbs protocol is cost-effective and more efficient for generating more potent T cells, thereby expecting a better therapeutic outcome.

Keywords: Adoptive cellular therapy, Immobilized antibodies, Lymphocyte activation, Memory phenotype

A synergistic approach to treating pancreatic ductal adenocarcinoma through the combination of radiotherapy and immunotherapy: a systematic review

Soheil Sadr[1], Elahe Roohbakhsh Amoli Moghadam[1], Hassan Borji[2]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Radio-immunotherapy works by targeting cancer cells with radiolabeled antibodies, which are specifically designed to bind to receptors on the surface of cancer cells. The radiation emitted by the radionuclides damages the cancer cells, leading to their death. The cytotoxicity of the radionuclides is due to their emission of α or β particles, which have high linear energy transfer and short ranges, making them highly effective in killing cancer cells. This study aims to investigate previous studies related to radiotherapy and immunotherapy for enhanced treatment of Pancreatic Ductal Adenocarcinoma (PDAC).

Methods: The study searched nine different databases, namely PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs, for articles published between January 2000 and January 2023 on the combined use of radiotherapy and immunotherapy to treat pancreatic ductal adenocarcinoma. Out of the articles found, twenty were selected for the study based on having complete abstracts. The data from these articles were extracted and analyzed using R version 4.2.1, an artificial intelligence software. The analysis was conducted on interconnected papers.

Results: In pancreatic ductal adenocarcinoma, radioimmunotherapy may be particularly effective due to the presence of certain receptors on the surface of cancer cells that can be targeted with radiolabeled antibodies. For example, the receptor for gastrin-releasing peptide (GRP) is highly expressed in PDAC, making it an attractive target for radioimmunotherapy. Other potential targets include Mesothelin, which is overexpressed in PDAC, and carcinoembryonic antigen (CEA), which is expressed in various cancers, including PDAC.

Conclusion: In conclusion, radioimmunotherapy using α- or β-emitting radionuclides has emerged as a promising treatment option for PDAC. Developing selective and cytotoxic radioimmunoconjugates targeting specific receptors expressed in PDAC can potentially overcome the limitations of conventional treatments and improve patient outcomes.

Keywords: Cancer, pancreatic ductal adenocarcinoma, Immunotherapy, Radio-immunotherapy

The Inhibitory effect of the combination of Immune checkpoint inhibitor (CTLA-4) and CD73 inhibitor on MDSCs in the growth of lung cancer cells

Elham Baghbani[1], Saeed Noorolyai[1], Behzad Baradaran[1]

1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Despite significant advances in the introduction of new treatments, the prognosis for lung cancer is still poor, and only 15% of lung cancer patients survive 5 years after diagnosis. Immunosuppression induced by MDSCs is largely attributed to CD39 and CD73 molecules, which are major tumor escape factors in antitumor therapies in lung cancer. Overexpression of CTLA-4 is often associated with chronic inflammation and cancer. CTLA-4 in the tumor microenvironment may play a role in the dysregulation of the immune response in cancer. It seems that targeting these molecules can have a significant effect in enhancing cancer treatment.

Methods: The frequency of MDSCs in the blood of lung cancer patients and healthy donors was evaluated by flow cytometry. Using the MACS technique, MDSCs and T lymphocytes were isolated from the blood samples and were co-cultured with the A549 cell line and treated with CD73 inhibitor and CTLA-4 mAb. Then the frequency of MDSCs, the differentiation into regulatory T lymphocytes, and the apoptosis rate in A549 cells were evaluated. Also, the division and proliferation of T lymphocytes using CSFE color were investigated by flow cytometry.

Results: Our study determined that MDSCs are present in the blood of healthy donors with a very low percentage, but a significant increase was observed in patients with lung cancer. The results of this study showed that following the inhibition of CD73 and CTLA-4, the percentage of MDSCs was significantly decreased, and the frequency of regulatory T lymphocytes was decreased in both healthy and diseased groups when both inhibitions were used at the same time. Also, the treatment induced the proliferation of T lymphocytes. Apoptosis was induced in A549 cells following co-culture and treatment with CD73 and CTLA-4 inhibitors.

Conclusion: In general, the simultaneous inhibition of CD73 and CTLA-4 can disrupt the immunosuppressive network in the tumor microenvironment and can be considered an effective therapeutic strategy for lung cancer immunotherapy alone or in combination with other immunotherapies.

Keywords: Lung cancer, CD73, CTLA-4, MDSC, immunotherapy, combination immunotherapy

The sensitivity of chronic lymphoblastic leukemia cells to therapy is enhanced by the targeted silencing of NRF2 by nanoparticles.

Farhad Jadidi-Niaragh[1], Atefeh Khodakarami[2]

1. Tabriz University of Medical Sciences, Tabriz, Iran.
2. Immunology Research Center, Tabriz, Iran.

Background: Targeting influential factors in resistance to chemotherapy is one way to increase the effectiveness of chemotherapeutics. The nuclear factor erythroid 2-related factor 2 (Nrf2) pathway overexpresses in chronic lymphocytic leukemia (CLL) cells and appears to have a significant part in their survival and chemotherapy resistance. Here we produced novel nanoparticles (NPs) specific for CD20-expressing CLL cells with simultaneous anti-Nrf2 and cytotoxic properties.

Methods: Chitosan lactate (CL) was used to produce the primary NPs which were then respectively loaded with rituximab (RTX), anti-Nrf2 siRNAs, and Cyclophosphamide to prepare the final version of the NPs (NP-Nrf2_siRNA-CP). All interventions were done on both peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells (BMNCs).

Results: NP-Nrf2_siRNA-CP had satisfying physicochemical properties, showed controlled anti-Nrf2 siRNA/CP release, and were efficiently transfected into CLL primary cells (both PBMCs and BMNCs). NP-Nrf2_siRNA-CP were significantly capable of cell apoptosis induction and proliferation prevention marked by respectively decreased and increased anti-apoptotic and pro-apoptotic factors. Furthermore, the use of anti-Nrf2 siRNA was corresponding to the elevated sensitivity of CLL cells to CP.

Conclusion: Our findings imply that the combination therapy of malignant CLL cells with RTX, CP, and anti-Nrf2 siRNA is a novel and efficient therapeutic strategy that was capable of destroying malignant cells. Furthermore, the use of NPs as a multiple-drug delivery method showed fulfilling properties; however, the need for further future studies is undeniable.

Keywords: Chronic lymphocytic leukemia (CLL), NRF2, Cyclophosphamide, Rituximab, chemo-resistance

Tim3 as a therapeutic and prognostic marker in colorectal cancer: relationships with sidedness, clinicopathological parameters, and survival

Zahra Mokhtari[1]

1. Isfahan University of medical science, Isfahan, Iran

Background: Colorectal cancer (CRC) is a heterogeneous disease that complicates predicting a patient's prognosis and response to treatment. CRC prognosis is influenced by the tumor microenvironment (TME). The immune system is a critical component of the TME. T-cell immunoglobulin and mucin-domain containing-3 (Tim3) is an inhibitory immune checkpoint that regulates immune response and may provide prognostic power. However, the effect of its expression on the CRC prognosis remains unclear. This study aimed to investigate the prognostic value of the Tim3 expression.

Methods: 136 patients with CRC who underwent curative surgery were enrolled in this study. Immunohistochemical staining was performed for Tim3 and its expression was evaluated in the center of the tumor (CT), invasive margin (IM), and adjacent normal-like tissue.

Result: High Tim3 expression in CT was associated with higher M stage (M1) (in left-sided CRCs) (P< 0.05). In addition, it was associated with poor OS in the total cohort (HR= 1.769, 95% CI= 1.050-2.980, *P*= 0.032) and left-sided CRCs (HR= 2.064, 95% CI= 1.063-4.007, P= 0.032) and was an independent prognostic factor for CRC patients (Multivariable: HR= 1.799, 95% CI= 1.057-3.065, P= 0.031).

Conclusion: Our findings suggest that the clinicopathological and prognostic significance of Tim3 depends on the primary tumor sides. We also showed that Tim3 could act as a prognostic factor and therapeutic target in CRC.

Keywords: Colorectal cancer, Immune checkpoint proteins, Immunohistochemistry, prognosis, Tim3 protein

Clinical Laboratory Immunology

Assessment of the Correlation among Serum Protein Electrophoresis Bands in Clinical Interpretations: A Case-Series Study

Mahdi Shooraj[1], Hossein Mokhtari[2], Maysam Rezapour[2], Seyedeh Farzaneh Jalali[3], Mohammad Reza Mahdavi Amiri[4], Seyed Ehsan Enderami[5], Hadi Hassannia[2]

1. Student Research Committee, Amol School of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Paramedicine, Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
3. Department of Hematology and Medical Laboratory Sciences, Faculty of Allied Medicine, Kerman University of Medical Sciences, Kerman, Iran.
4. Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
5. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

Background: Serum Protein Electrophoresis (SPE) has been one of the routine laboratory tests in the diagnosis of many disorders such as hepatitis, cirrhosis, gammopathies, leukemias, malignancies, nephrotic syndrome, and inflammatory diseases. Nevertheless, interpretation of the results is occasionally challenging for physicians. Thus, the present study aimed to clarify the test results and clinical condition of abnormal patients.

Methods: In this study, 785 electrophoresis results were collected from Mazandaran laboratories. Participants were categorized based on their age group, gender, and the alterations in the pattern of their SPE bands. Formerly, the correlation among SPE bands was analyzed via GraphPad Prism (v9.0.0) software.

Results: A total of 785 samples including 292 males (37.2%) and 493 females (62.8%) from 10 age groups were comprised in this study. Data analysis revealed that there is a correlation among SPE bands. For instance, albumin and age had a converse relation. On the other hand, alpha-1 and age were directly correlated. Accordingly, the weakened protein expression in older ages, due to liver failure or related diseases was approved in our study. Moreover, clinical laboratory data of selected patients including other confirmatory biochemical and hematological tests were followed and the interpretations of suspected disorders were evaluated.

Conclusion: This study showed that the most significant change in the pattern of SPE bands belonged to albumin, which represented a declining trend. This reduction pattern was along with the lessening beta-1 band. Also, the most increase was observed in the gamma band, indicating the prevalence of gammopathies toggled with inflammatory diseases. Similarly, results showed that the albumin and Alpha-2 bands diminish by increasing inflammatory disorders mostly in older ages. Respectively, the outcome approved that laboratory data concerning the correlation among SPE bands besides other biomarkers is helps clarify the clinical condition of the patients for a more confident diagnosis.

Keywords: Laboratory, Serum Protein Electrophoresis, Serum proteomics, and Clinical Interpretations

Cold Atmospheric Plasma increases antitumor CD8+ T cells proliferation in Chronic Lymphocytic Leukemia

Mina Alimohammadi[1], Monireh Golpour[2], Alireza Rafiei[2]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Mazandaran University of Medical Sciences, Mazandaran, Iran.

Background: Nowadays Many cancer types, including Chronic Lymphocytic Leukemia (CLL), are being treated with therapeutic strategies focused on enhancing T cells' antitumor function as a result of improving the patient's immune system. Cold atmospheric plasma (CAP) is one way of producing ROS that induces apoptosis, necrosis, and autophagy in cancer cells, as well as reducing cancer cell growth and boosting the sensitivity of chemotherapy-resistant cancer cells to particular medications. In this research, we aimed to see how CAP medication affected the restoration of exhausted CD8+T cells and their proliferation in CLL patients and healthy participants.

Methods: The in vitro findings showed that CAP treatment significantly elevated MDA and RNS levels in the supernatant of isolated CD8+T cells from both healthy individuals and CLL patients at four-time points of 45, 60, 90, and 120 s when compared to the negative control.

Results: CAP had no cytotoxic effect on CD8+T cells isolated from CLL patients, and it inhibited apoptosis in CD8+T cells after 45 and 60 seconds of CAP exposure. This confirms that CAP therapy can boost the proliferation of CD8+ T lymphocytes in CLL patients. We also showed that the SI in CLL patients treated with CAP was significantly higher in both co-culture with CD19+B cells (p=0.0027) and without (p=0.0134) compared to the untreated groups.

Conclusion: Overall, the findings of this study indicated that ROS acts as a crucial messenger in CLL patients' T lymphocytes to enhance cell proliferation. More research on T cell activation, cytokine generation, and exhaustion is needed to provide a more accurate and comprehensive knowledge of the use of CAP in CLL immunotherapy.

Keywords: Chronic lymphocytic leukemia (CLL), CD8+ T cells, CD19+B cells, MDA, nitric oxide, apoptosis, plasma medicine

Comparison the effects of exosomes derived from human serum, human cord blood serum, and fetal bovine serum (FBS) on peripheral blood mononuclear cell proliferation and nitric oxide production.

Ali Al-Faham[1], Saleh Noor[2], Farshid Zamanni[2], Seyed Mahmoud Masiha Hashemi[2]

1. University of kufa
2. Shahid Beheshti University of medical science, Tehran, Iran.

Background: Exosomes as nanovesicles of biological origin have attracted the attention of researchers in recent years. Until now, the effects of these small vesicles (30-150 nm) have been studied in many biological processes, including wound healing, changes in the inflammatory state, and changes in the function of the immune system. Some studies have also used exosomes as drug carriers in the in vivo environment. Exosomes have characteristics that make them suitable for such studies. For example, they have a very low antigenic property and their phospholipid bilayer membrane is similar to normal cells. The contents of exosomes are derived from their generative cells. In fact, they carry many proteins in the cytoplasm and endosome of the cell that secreted them. In fact, according to the nature of exosomes, their effects depend to a large extent on the cells that secrete them. Since almost all the cells of the human body and other mammals are in contact with the blood and release their secretions into the blood, one of the rich sources of exosomes is the circulatory system of mammals and their serum. Knowing the characteristics and effects of different serum exosomes on different cell lines cannot be ignored. As the most important defense barrier of the body, the immune system consists of cells that are directly in contact with the blood and are affected by the exosomes in the serum. Based on this, in this study, we intend to investigate and compare the effects of exosomes isolated from the blood serum of healthy adults, umbilical cord blood serum, and FBS on the activity of monocytes and the proliferation of lymphocytes.

Methods: In order to investigate the effects of exosomes isolated from different sources, including the blood serum of healthy adults, umbilical cord blood serum, and also FBS, on the immune responses of monocytes and lymphocytes, first a sufficient amount of exosomes from the mentioned sources was extracted. To confirm the identity of these exosomes, they went through several steps and tests including BCA to determine protein concentration, DLS to determine the size and photography with SEM and TEM electron microscope. In the next step, in order to obtain lymphocyte and monocyte populations, blood was collected from healthy volunteers, and peripheral blood mononuclear cells were extracted using Faicol. In the following, based on the adhesion property of monocytes to the cell culture plate, we separated lymphocytes and monocytes from each other. Then we cultured the obtained lymphocytes and monocytes in different groups and treated the lymphocytes with PHA and exosome and the monocytes with LPS and exosome according to the studied groups. In the end, the proliferation of lymphocytes was evaluated using the MTT kit and the activity of monocytes was evaluated using the NO assay kit, and the phagocytosis ability of yeast was evaluated in them.

Results: In the presence of PHA substance, exosomes isolated from all three sources of blood serum of healthy people, FBS, and umbilical cord blood serum caused a significant decrease in the proliferation of lymphocytes compared to the positive control group that was treated only by PHA. The intensity of inhibition in the group receiving umbilical cord serum exosome, the Ex-UCBS+PHA group, was higher than the other two groups and had a significant difference with them. In the presence of LPS as a factor stimulating the immunological activity of macrophages, a group of monocytes that received umbilical cord blood exosomes had a significant decrease in NO production and yeast phagocytosis compared to the control group that did not receive exosomes. In our study, it was observed that FBS-derived exosomes also decreased NO production by monocytes, however, the inhibitory power of NO production by umbilical cord exosomes was significantly higher than in this group. Exosomes derived from the blood serum of healthy people did not affect the production of NO by monocytes, however, unlike exosomes derived from FBS, they decreased the phagocytosis power of monocytes in our study.

Conclusion: The exosomes derived from the umbilical cord blood serum have a high ability to inhibit lymphocyte proliferation compared to the exosomes isolated from serum and FBS. In addition, these exosomes cause a further decrease in NO production and the phagocytosis power of monocytes.

Keywords: Exosome, cord blood serum, immune system, immunomodulation, peripheral blood mononuclear cells, monocyte, lymphocyte

Correlations of para-clinical and laboratory factors with the severity of Covid-19 in hospitalized patients during the pandemic period

Mansoor Rajaie[1], Alireza Andalib[2], Farzin Khorvash[3]

1. Biochemistry Department, Isfahan Medical School-Isfahan University of Medical Sciences, Isfahan, Iran.
2. Immunology department, Isfahan Medical School-Isfahan University of Medical Sciences, Isfahan, Iran.
3. Infectious disease department, Isfahan Medical School-Isfahan University of Medical Sciences, Isfahan, Iran.

Background: In order to confirm, follow up and evaluate the covid-19 disease, various tests would be requested for hospitalized patients, to evaluate the general and vital condition of the patients. The complications of inflammation caused by the respiratory virus covid-19 are monitored duration of hospitalization. With this purpose, various factors of para-clinic laboratory tests would be performed. The purpose of the study is to evaluate the number of inflammatory factors measured for patients in the laboratory and the power of prediction of them with the severity of clinical complications of patients.

Methods: the relationship of Blood cell counts was analyzed with laboratory factors such as ESR, CRP, Cr, ALT, LDH, D-Dimer, Ferritin, O2 saturation, long involvements, and duration of hospitalization. The Pearson correlation statistical software was used for analysis.

Results: The factors O2 saturation and D-Dimer show a negative correlation (p=0.0001, R=-0.481) and with ALT and duration of hospitalization are too. And there was a negative statistical correlation between CRP, LDH, Cr, and lymphocyte counts of patients. A positive statistical correlation was obtained between the LDH, CRP, ALT, and D-dimer with the length of the hospitalization period as well as the percentage of pulmonary involvement. Specifically, the length of hospitalization and the percentage of pulmonary involvement were positively correlated.

Conclusion: The statistical analysis with Pearson's correlation of many factors measured in the laboratory has a significant positive and negative correlation with a constant trend, which could be used for the prediction of other parameters that are statistically justified and acceptable.

Keywords: LDH, CRP, ALT, D-dimer, hospitalization

Diagnosing Celiac Disease: Common Methods

Nazanin Moghaddam[1], Sina Moghaddam[2], Maryam Meskini[2]

1. Laboratory of Medicinal Chemistry, Department of Clinical Research, Pasteur Institute of Iran, Tehran, Iran
2. Department of Mycobacteriology and Pulmonary Research, Pasteur Institute of Iran, Tehran, Iran

Background: Gluten consumption is the cause of celiac disease (CD), an autoimmune disease associated with a genetic predisposition. The wheat family includes wheat, rye, barley, and oats, which are in the aveneae family. Gluten refers technically to peptides present in wheat that activate CD; gluten is used as a general description of the trigger. Peptides in barley and rye are able to activate the disease. The avenins in oats, however, rarely cause disease because they contain peptides. As a result of deamidating gliadin peptide, high-affinity binding can be established with celiac-related human leukocyte antigen (HLA) peptides (DQ2 or DQ8) of cells that are antigen-bearing. As a result, CD4+ Th cells are activated. A study has shown that tTG stimulates gluten-specific T-cell immune responses through the deamidation of specific toxic epitopes. In addition, special attention has recently been paid to intestinal epithelial damage caused by CD8+CD4 intraepithelial lymphocytes. This is why celiac disease predisposition requires HLA-DQ2 (95% of celiac patients) or HLA-DQ8 (5%).

Methods: Celiac disease and standard diagnostic methods have been extensively researched in the literature. Various keywords (routine diagnosis, Celiac disease, Gluten, and routine diagnostic methods) were used to search both international databases (Cochrane Library, Web of Science, Google Scholar, MEDLINE, PubMed, Science Direct, Scopus, Academic Search, Journal Storage, and Scientific Information Database). A selection of English-language articles published between 2010 and 2023 was considered.

Results: CD or gluten-sensitive intestinal damage has become more prevalent in Western and developing countries. Serological diagnostic tests have led to early diagnosis of CD due to the increase in the number of tests. In most European countries as well as in North, South, Asia, India, and the Middle East, a variety of diagnostic tests were conducted in order to investigate the prevalence and occurrence of CD. Most of these regions have a high prevalence of CD, according to their findings. In contrast to previous reports, a higher prevalence than reported was found for this disease.

Conclusion: The development of new diagnostic methods has led to the development of low-cost devices with low storage requirements, including the lateral flow immunoassay, a simple immunochromatographic technique used to perform rapid diagnostic tests, typically made from nitrocellulose or paper-based porous membranes. Symptoms of celiac disease can be detected quickly with these tests. Since the serum is made up of natural ingredients, it moves easily.

Keywords: Celiac, Gluten, Diagnostic methods

Down-regulation of miR-4443, miR-572, and miR-150-5p in serum of breast cancer patients as a potential diagnostic biomarker: A study based on bioinformatics methods

Amirhossein Mardi[1], Davar Amani[1]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Breast cancer, which affects more than 500,000 women yearly, is the most prevalent invasive cancer in women and the leading cause of cancer-related fatalities. The current study intends to develop a reliable panel of microRNAs (miRNA) to accurately detect patients with breast cancer in clinical settings.

Methods: The miRNAs that were elevated in the interstitial fluid of the tumor were chosen for this study using bioinformatics methods and data achieved from the GEO database. Then, using the Real-time PCR technique, the expression levels of 3 potential miRNAs on serum samples from 26 breast cancer patients and 15 healthy controls, as well as 14 tumor tissue samples and 14 adjacent normal tissue samples, were examined in the laboratory phase. Then sensitivity and specificity of candidate miRNAs were evaluated through the ROC curve. GraphPad Prism was used for the data analysis.

Results: In this study, we demonstrated that miR-4443, miR-572, and miR-150-5p expression levels were considerably lower in breast cancer patients' serum when compared to healthy controls. Additionally, tumor tissue exhibits significantly lower levels of miR-4443 and miR-150-5p expression relative to non-tumor adjacent tissue.

Conclusion: Based on our findings, serum miRNA measurements from tumors are an essential method for the blood-based diagnosis of breast cancer in Iranian women. We discovered that the diagnostic biomarkers miR-4443, miR-572, and miR-150-5p are highly sensitive and specific.

Keywords: Breast cancer, early detection, microRNA, Bioinformatic analysis

Evaluation of the progesterone effect on membrane progesterone receptor (mPR-β) expression on NALM6 cells

Narges Fallahi[1], Mitra Rafiee[2], Vahid Bagheri[2]

1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran.
2. Cellular and Molecular Research Center, Department of Immunology, Birjand University of Medical Sciences, Birjand, Iran.

Background: Acute lymphoblastic leukemia (ALL) is an uncommon type of bone marrow and blood cancer that rapidly progresses without treatment. A number of studies have revealed progesterone (P4) efficiency in the treatment of some tumors. Membrane progesterone receptor beta (mPR-β) may be responsible for enhancer or inhibitor action in cell growth in different tumors. This study determined the P4 effect on mPR-βexpression in NALM6 cells.

Methods: The mPR-β expression on NALM6 cells was determined using flow cytometry. The cells were exposed to different concentrations of P4 (10-100 µM) at 48 and 72 h. Then, cell survival was assessed using an MTT assay. Finally, the rate of mPRβexpression in the cells was evaluated after treatment with P4 (10 and 20 µM) at 48 and 72 h, respectively using flow cytometry.

Results: our result showed that mPRβwas expressed on NALM6 cells. P4 significantly inhibited the growth of tumor cells in time and concentration-dependent manners and reduced mPRβexpression at 48 and 72 h.

Conclusion: The decrease in mPRβexpression after the P4 effect, may be considered a promising therapeutic potential in ALL patients due to its remarkable efficiency in killing tumor cells.

Keywords: Acute lymphoblastic leukemia, Progesterone, Progesterone receptor, mPR-β

Evolution of CD Markers as Biomarkers for SLE Disease Activity

Bita Poorabbas Pasha[1], Bahar Khaelghi Hozhabrasa[1], Golnaz Khodayari[1], Amir Gholamzad[1], Omid Moeini[1], Mehrdad Gholamzad[2]

1. Department of Laboratory Medicine, Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
2. Department of Microbiology and Immunology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by various clinical manifestations and immune dysregulation. CD (cluster of differentiation) markers are commonly used to identify specific cell populations and monitor disease progression in SLE patients.

Methods: We conducted a retrospective analysis of 100 SLE patients at a single center to investigate the evolution of CD markers over time. Patients with active disease or in remission were included. CD marker expression was measured by flow cytometry and analyzed in relation to disease activity, as measured by the SLEDAI.

Results: We found that the expression of several CD markers differed significantly between patients with active disease and those in remission. For example, CD19 and CD20 were significantly decreased in patients with active disease compared to those in remission, whereas CD38 was significantly increased in active disease. In addition, CD markers were associated with disease severity, as measured by the SLEDAI, with higher expression of CD38, CD11c, and CD4+CD28- T cells associated with more severe disease activity.

Conclusion: The study concludes that monitoring the evolution of CD markers can be a valuable approach for evaluating SLE disease activity and guiding personalized treatments. Decreased expression of CD19 and CD20 and increased expression of CD38 can be useful biomarkers for identifying SLE patients at risk of active disease. Further research is required to confirm the validity of these biomarkers and investigate their underlying mechanisms

Keywords: "CD Marker", 'SLE", "Diagnosis"

Impact of Interleukins on Asthma: Insights from Biomarker Evaluation in Adult Patients

Golnaz Khodayari[1], Bita Pourabbas Pasha[1], Bahar Khaleghi Hozhabrasa[1], Amir Gholamzad[1], Mehrdad Gholamzad[2]

1. Department of Laboratory Medicine, Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
2. Department of Microbiology and Immunology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

Background: Asthma is a chronic respiratory disease that affects millions of people worldwide. Interleukins (ILs) play a key role in the pathogenesis of asthma, serving as important mediators for airway inflammation and hypersensitivity. In this study, we aimed to investigate the evolution of ILs in 100 adult asthma patients.

Methods: Blood samples were collected from the patients at different time points, including before and after treatment initiation. The levels of several ILs, including IL-5, IL-13, and IL-17, were measured using enzyme-linked immunosorbent assay (ELISA).

Results: In this study, it was found that IL-5 and IL-13 levels were higher in asthma patients and positively correlated with asthma severity. However, elevated levels of IL-17 were observed only in a subgroup of patients. Following treatment initiation, there was a noticeable reduction in the levels of IL-5 and IL-13 in most patients. This suggests that IL-5 and IL-13 could be helpful biomarkers for monitoring asthma severity and treatment response.

Conclusion: this study provides new insights into the evolution of ILs in asthma patients and highlights the potential use of ILs as biomarkers for monitoring disease progression and treatment response.

Keywords: "Asthma", "Interleukin", "Cytokine", "ELISA"

Increased expression of Integrin Beta 3 in leukemia by cold atmospheric plasma and cold atmospheric plasma active medium

Mina Alimohammadi[1], Monireh Golpour[2], Alireza Rafiei[2]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Mazandaran University of Medical Sciences, Tehran, Iran.

Background: Chronic lymphocytic leukemia (CLL) is the most prevalent hematological cancer, with various medical interventions. In recent decades, cold physical plasma has been seen as a viable method in cancer therapy.

Methods: The goal of this study was to see how cold plasma or cold plasma-activated medium (CAM) affected integrin beta 3 (ITGB3) expression in CLL patients' whole blood.

Results: Both direct plasma and indirect CAM therapy enhanced lipid peroxidation in the whole blood of CLL patients and healthy participants, according to the findings. Furthermore, following 48 hours of cold plasma or CAM therapy, the metabolic activity of PBMCs was reduced. However, cold plasma and CAM therapy boosted ITGB3 expression in CLL patients' PBMCs compared to untreated and healthy controls. The current study identifies cold plasma components that may decrease PBMC metabolic activity and increase ITGB3 expression in CLL patients, confirming the idea that the effect of cold plasma may not be limited to influencing hematologic tumor cells and may extend to other immune cells such as NK cells.

Conclusion: Additional cellular and molecular research is required to assess the effect of plasma or CAM on NK cell activation in CLL patients.

Keywords: Cold atmospheric plasma, chronic lymphocytic leukemia, NK cells, Cytotoxicity, ROS

Interleukin Levels in Pulmonary Diseases

Tina Kabipour[1], Amir Gholamzad[1], Mohammad Matin Nourikhani[1], Mehrdad Gholamzad[2]

1. Department of Laboratory Medicine, Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
2. Department of Microbiology and Immunology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.

Background: Interleukins (ILs) are cytokines that play a vital role in the immune response to respiratory infections, pulmonary inflammation, and lung injury. The aim of this study was to measure the levels of IL-1β, IL-6, IL-8, and IL-10 in 100 patients with different pulmonary diseases.

Methods: Blood samples were obtained from 100 patients with pulmonary diseases, including chronic obstructive pulmonary disease, asthma, idiopathic pulmonary fibrosis, and lung cancer, as well as 50 healthy controls. The serum levels of IL-1β, IL-6, IL-8, and IL-10 were measured using enzyme-linked immunosorbent assay (ELISA) techniques.

Results: The results showed that the levels of IL-1β, IL-6, and IL-8 were significantly higher in patients with pulmonary diseases than in healthy controls ($p < 0.05$). Among the different pulmonary diseases, IL-6 levels were the highest in patients with lung cancer, while IL-8 levels were significantly higher in patients with asthma and idiopathic pulmonary fibrosis. IL-10 levels were also significantly elevated in patients with pulmonary diseases compared to the healthy controls.

Conclusion: In conclusion, our findings suggest that the levels of IL-1β, IL-6, IL-8, and IL-10 are elevated in patients with different pulmonary diseases. These cytokines may serve as useful biomarkers for the early diagnosis, prognosis, and monitoring of pulmonary diseases. Further studies are warranted to evaluate the clinical utility of these cytokines in the management of pulmonary diseases

Keywords: "Interleukins", "Pulmonary Diseases", "ELISA"

Interleukins as potential diagnostic and therapeutic targets in cardiovascular disease

Bahar Khaleghi Hozhabrasa[1], Bita Pourabbas Pasha[1], Golnaz Khodayari[1], Amir Gholamzad[1], Mehrdad Gholamzad[2]

1. Department of Laboratory Medicine, Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
2. Department of Microbiology and Immunology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.

Background: Inflammation is known to play a crucial role in the development and progression of cardiovascular disease (CVD). Interleukins are immune mediators that contribute to the regulation of inflammatory responses and immune cell function, making them a potential therapeutic target for managing CVD.

Methods: Blood samples were collected from 50 patients with CVD and the serum levels of three interleukins (IL-1β IL-6, and IL-18) were measured using ELISA. The results were compared to those of healthy controls.

Results: The study found that CVD patients had significantly higher levels of IL-1β IL-6, and IL-18 compared to healthy controls. Additionally, the levels of these interleukins were positively correlated with the severity of CVD.

Conclusion: These findings suggest that interleukins may play a crucial role in the pathogenesis of CVD and could be potential diagnostic and therapeutic targets for managing this disease. Further research is needed to explore the effectiveness of targeting these immune mediators in the treatment of CVD

Keywords: CVD, Interleukin, Cytokine, ELISA

Interleukins in Seminal Plasma of Male Infertility Patients

Seyed Armit Hosseini[1], Omid Moeini[1], Mohammad Matin Nourikhani[1], Amir Gholamzad[1], Mehrdad Gholamzad[2]

1. Department of Laboratory Medicine, Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
2. Department of Microbiology and Immunology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

Background: Male infertility is a global problem with an undefined etiology. Recent research suggests that inflammation and immune dysfunction may play a role. This study aimed to measure Interleukin-1β Interleukin-6, and Interleukin-8 in the seminal plasma of 100 male infertility patients and correlate the findings with clinical data.

Methods: The study was conducted on hundred male infertility patients who attended a tertiary infertility center. Seminal plasma was collected and ELISA was used to measure Interleukin-1β Interleukin-6, and Interleukin-8 levels. Clinical data, including age, duration of infertility, semen parameters, and histopathological evidence of testicular biopsies among others, were collected.

Results: Results showed that Interleukin-1β Interleukin-6, and Interleukin-8 are present in the seminal plasma of male infertility patients. Interleukin-1β correlated with sperm motility, Interleukin-6 correlated with sperm DNA fragmentation, and Interleukin-8 correlated with oxidative stress, inflammation, and abnormal sperm parameters.

Conclusion: Measuring Interleukin levels in male infertility patients may provide insight into the pathogenesis of male infertility and guide treatment strategies. However, further large-scale studies are needed to validate these findings

Keywords: Interleukins, Male infertility, seminal plasma, ELISA

Investigating the Fluctuations of Albumin Levels in Suspected Inflammatory Patients Using Serum Protein Electrophoresis Assay in Mazandaran Province, during 2022-2023

Sepideh Khansari[1], Mahdi Shooraj[1], Seyedeh Farzaneh Jalali[2], Mohammad Reza Mahdavi Amiri[3], Seyed Ehsan Enderami[4], Hossein Mokhtari[4], Maysam Rezapour[5], Hadi Hassannia[4]

1. Student Research Committee, Amol School of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Hematology and Medical Laboratory Sciences, Faculty of Allied Medicine, Kerman University of Medical Sciences, Kerman, Iran.
3. Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
4. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
5. Department of Paramedicine, Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran.

Background: Serum Protein Electrophoresis (SPE) is a widely available and inexpensive laboratory test that examines several protein fractions including Albumin, Alpha 1, Alpha 2, Beta 1, Beta 2, and Gamma globulins. The examination of albumin is useful in the assessment of hepatic insufficiency, renal failure, protein loss, chronic infection, and gammopathies in addition to dehydration. This study aimed to evaluate the frequency of patients with abnormal ranges of albumin referred to the laboratory.

Methods: In this study, 785 SPE results were collected from Mazandaran laboratories. Participants were classified by their age group and gender. Their electrophoretic bands were evaluated with an emphasis on the albumin band. Formerly, the correlation among SPE bands was analyzed via GraphPad Prism (V9.0.0) software.

Results: A total of 785 samples including 292 males (37.2%) and 493 females (62.8%) from 10 age groups were included in this study. According to data analysis, the frequency of hypoalbuminemia and hyperalbuminemia was 38% and 2.5% respectively. Moreover, the results showed that there was a converse correlation among albumin and age, alpha-2 band, beta-2 band, and gamma.

Conclusion: This study revealed an exceedingly high prevalence of hypoalbuminemia which might indicate the prevalence of liver and renal failure along with chronic inflammation in examined population. Hence, assessment of albumin level via SPE test is appropriate for primary screening in the diagnosis of many inflammatory diseases.

Keywords: Albumin, Serum Protein Electrophoresis, Hypoalbuminemia

Investigating the Fluctuations of Alpha Globulin Levels in Suspected Inflammatory Patients Using Serum Protein Electrophoresis Assay in Mazandaran Province, during 2022-2023

Omidreza Samdaliri[1], Mahdi Shooraj[1], Seyedeh Farzaneh Jalali[2], Mohammad Reza Mahdavi Amiri[2], Seyed Ehsan Enderami[4], Hossein Mokhtari[4], Maysam Rezapour[5], Hadi Hassannia[4]

1. Student Research Committee, Amol School of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Hematology and Medical Laboratory Sciences, Faculty of Allied Medicine, Kerman University of Medical Sciences, Kerman, Iran
3. Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran
4. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
5. Department of Paramedicine, Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran.

Background: Serum Protein Electrophoresis (SPE) is a widely available and inexpensive laboratory test that examines several protein fractions, including Albumin, Alpha 1, Alpha 2, Beta 1, Beta 2, and Gamma globulins. The examination of alpha bands is useful in the assessment of hepatic insufficiency, renal failure, protein loss, chronic inflammation in addition to malnutrition. The aim of this study was to evaluate the frequency of patients with abnormal ranges of alpha globulins referred to the laboratory.

Methods: In this study, 785 SPE results were collected from Mazandaran laboratories. Participants were classified by their age group and gender. Their electrophoretic bands were evaluated with an emphasis on the alpha band. Formerly, the correlation among SPE bands was analyzed via GraphPad Prism (v9.0.0) software.

Results: A total of 785 samples, including 292 males (37.2%) and 493 females (62.8%) from 10 age groups were included in this study. According to data analysis, the frequency of high alpha-1 globulin and high alpha-2 globulin was 21.9% and 22.5% respectively. Additionally, the results showed that there was a direct correlation between age and both alpha-1 and alpha-2 bands. On the other hand, the alpha-2 band had a converse correlation with albumin, beta-2, and gamma bands, while the alpha-1 band had a direct correlation with the beta-2 band.

Conclusion: This study revealed that high alpha-1 globulin was more prevalent than low alpha-1 globulin, which might indicate the prevalence of inflammatory diseases along with cancers in examined population. Hence, assessment of alpha globulins level via SPE test is appropriate for primary screening in the diagnosis of many inflammatory diseases.

Keywords: Alpha globulin, Serum Protein Electrophoresis, Inflammation

Investigating the Fluctuations of Beta Globulin Levels in Suspected Inflammatory Patients Using Serum Protein Electrophoresis Assay in Mazandaran Province, during 2022-2023

Fateme Malekinezhad[1], Mahdi Shooraj[1], Seyedeh Farzaneh Jalali[2], Mohammad Reza Mahdavi Amiri[3], Seyed Ehsan Enderami[4], Hossein Mokhtari[4], Maysam Rezapour[5], Hadi Hassannia[4]

1. Student Research Committee, Amol School of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Hematology and Medical Laboratory Sciences, Faculty of Allied Medicine, Kerman University of Medical Sciences, Kerman, Iran
3. Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran
4. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
5. Department of Paramedicine, Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran

Background: Serum Protein Electrophoresis (SPE) is a widely available and inexpensive laboratory test that examines several protein fractions including Albumin, Alpha 1, Alpha 2, Beta 1, Beta 2, and Gamma globulins. The examination of beta globulin is useful in the assessment of hepatic insufficiency, renal failure, protein loss, chronic infection, and gammopathies in addition to dehydration. The aim of this study was to evaluate the frequency of patients with abnormal ranges of beta globulin referred to the laboratory.

Methods: In this study, 785 SPE results were collected from Mazandaran laboratories. Participants were classified by their age group and gender. Their electrophoretic bands were evaluated with an emphasis on the beta band. Formerly, the correlation among SPE bands was analyzed via GraphPad Prism (v9.0.0) software.

Results: A total of 785 samples, including 292 males (37.2%) and 493 females (62.8%) from 10 age groups were included in this study. According to data analysis, the frequency of high beta-1 globulin and high beta-2 globulin was 7.5% and 14.5% respectively. Additionally, the results showed that there was a direct correlation between age and the beta-2 band. On the other hand, the beta-1 band had a converse correlation with the gamma band, while the beta globulins had a direct correlation with alpha globulins.

Conclusion: This study revealed that high beta globulin was more prevalent than low beta globulin which might indicate the prevalence of inflammatory diseases along with liver failure in examined population. Hence, assessment of alpha globulins level via SPE test is appropriate for primary screening in the diagnosis of many inflammatory diseases.

Keywords: Beta globulin, Serum Protein Electrophoresis, Inflammation

Investigating the Fluctuations of Gamma Globulin Levels in Suspected Inflammatory Patients Using Serum Protein Electrophoresis Assay in Mazandaran Province, during 2022-2023

Arian Amini[1], Mahdi Shooraj[1], Seyedeh Farzaneh Jalali[2], Mohammad Reza Mahdavi Amiri[3], Seyed Ehsan Enderami[4], Hossein Mokhtari[4], Maysam Rezapour[5], Hadi Hassannia[4]

1. Student Research Committee, Amol School of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Hematology and Medical Laboratory Sciences, Faculty of Allied Medicine, Kerman University of Medical Sciences, Kerman, Iran
3. Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran
4. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
5. Department of Paramedicine, Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran

Background: Serum Protein Electrophoresis (SPE) is a widely available and inexpensive laboratory test that examines several protein fractions including Albumin, Alpha 1, Alpha 2, Beta 1, Beta 2, and Gamma globulins. Mostly, clinical interest is focused on the gamma region of the serum protein spectrum since, the examination of the Gamma band is useful in the assessment of chronic infections, Cirrhosis, Malignant lymphoma, Multiple myeloma, and Autoimmune diseases in addition to Hypogammaglobulinemia. The aim of this study was to evaluate the frequency of patients with abnormal ranges of gamma band referred to the laboratory.

Methods: In this study, 785 SPE results were collected from Mazandaran laboratories. Participants were classified by their age group and gender. Their electrophoretic bands were evaluated with an emphasis on the gamma band. Formerly, the correlation among SPE bands was analyzed via GraphPad Prism (v9.0.0) software.

Results: A total of 785 samples, including 292 males (37.2%) and 493 females (62.8%) from 10 age groups were included in this study. According to data analysis, the frequency of hypogammaglobulinemia and hypergammaglobulinemia was 4.1% and 23.7% respectively. Moreover, the results showed that gamma globulins had a converse correlation with albumin, alpha globulins, and beta globulins.

Conclusion: This study revealed an exceedingly high prevalence of hypergammaglobulinemia which might indicate the prevalence of gammopathies and multiple myeloma along with chronic inflammation in examined population. Hence, assessment of gamma globulin level via SPE test is appropriate for primary screening in the diagnosis of gammopathies and many inflammatory diseases.

Keywords: Gamma globulin, Serum Protein Electrophoresis, Gammopathy, Inflammation

Investigating the rapid diagnostic value of Cardiac Troponin I (cTnI) compared to the EIA method, for detecting early myocardial damage.

Parya Abdollahi[1], Mj Rasaee[2]

1. Department of Research and Development, Rojam Azma mfg, Alborz, Iran
2. Department of Medical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University. Tehran Iran

Background: Cardiac troponin I (cTnI) have been shown to be a highly sensitive and specific marker of myocardial cell injury. The purpose of this study was to investigate the diagnostic value of cTnI to determine whether it can be used for early diagnosis of myocardial damage. Cardiovascular diseases have a leading role in terms of morbidity, mortality, and disability of the population, causing significant socioeconomic damage to all countries of the world. This circumstance requires researchers to constantly seek new biomarkers and improve methods for determining existing biomarkers, and search for new therapeutic targets to improve diagnostic and treatment strategies. Recently, there have been some important changes in laboratory diagnostics of patients with acute coronary syndrome, due to the introduction into the routine practice of new high and ultrasensitive methods for the determination of biomarkers of injury, specific to cardiac muscle tissue, namely cardiac troponins.

Methods: One hundred adults with heart attack symptoms referred for EIA provided a whole blood/serum/plasma sample for testing. Patients were considered cTnI positive if EIA tests were positive. Whole blood/serum/plasma samples were collected from the same patients and were tested for cTnI rapid test. The cTnI one-step Troponin I test device is a qualitative, membrane-based immunoassay for the detection of cTnI in whole blood/serum/plasma. The membrane is pre-coated whit a capture reagent on the test line region of the test.

Results: The sensitivities and specificities of the Rojan Azma. Cardiac troponin I (cTnI) kits (rapid immunochromatography method) when compared with EIA diagnosis were, 98.5 %, and 98.5%, respectively.

Conclusion: The rapid diagnostic test of cardiac troponin I (cTnI) may be considered an alternative to EIA testing in the initial diagnosis of patients with heart attack symptoms who do not require Expensive and time-consuming tests. Whole blood/serum/plasma testing has the potential advantages of being simple to perform, relatively cheap, and samples can be submitted directly from primary care and performed with the least available hardware and trained personnel.

Keywords: Cardiac troponin I, Whole blood/serum/plasma, Rapid test, Immunoassay

Investigation of serum levels of ANA, Anti CCP, RF and ESR factors in patients with suspected rheumatoid arthritis

Mohammad Reza Ghanbarpour[1], Vida Yazdipour[1], Farimah Masoumi[2]

1. Student research Committee, School of Medicine, Tonekabon branch, Islamic Azad University, Tonekabon, Iran.
2. Department of Medical Immunology, School of Medicine, Tonekabon branch, Islamic Azad University, Tonekabon, Iran.

Background: Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic joint inflammation. The cause of rheumatoid arthritis is not known. This cause is related to a combination of hormonal or environmental and hereditary or genetic factors. One out of every hundred people will be affected by this disease in some way during their lifetime. Joint rheumatism, especially knee rheumatism, has a higher prevalence rate in women, and women suffer from this disease three times more than men. The symptoms of the disease include dryness, pain in the joints, weight loss, fever, fatigue, and weakness in different people. The aim of this study is to investigate the relationship between serum levels of ANA, Anti CCP, RF, and ESR factors in patients with suspected rheumatoid arthritis.

Methods: In this descriptive-cross-sectional study, 296 patients suspected of RA who were referred to the laboratories of Tonekabon City from April 1 to December 10, 2022, were investigated. ANA and Anti CCP on patient samples were evaluated using the ELISA method. RF was detected quantitatively by the latex agglutination method. Results were analyzed by student T-test using SPSS software.

Results: Among the 296 suspected rheumatoid arthritis patients, 226 (76%) were women and 70 (24%) were men, and most of the studied patients were between 50 and 70 years old. Among the 254 people whose ANA was measured, 5 people were positive. ESR was positive in 66 out of 243 people. RF test was positive in 63 cases out of 255 people. Anti-CCP was also positive in 14 out of 250 people.

Conclusion: The results have shown that most of the suspected RA patients are negative in terms of ESR, ANA, Anti CCP, and RF tests, and the relationship between RA symptoms and serology tests is more significant in older people than in younger people.

Keywords: _Rheumatoid arthritis, ANA, ESR, RF, Anti CCP_

Investigation of serum levels of ANA, Anti CCP, RF and ESR factors in patients with suspected rheumatoid arthritis

Mohammad Reza Ghanbarpour[1], Vida Yazdipour[1], Farimah Masoumi[2]

1. Student research Committee, School of Medicine, Tonekabon branch, Islamic Azad University, Tonekabon, Iran.
2. Department of Medical Immunology, School of Medicine, Tonekabon branch, Islamic Azad University, Tonekabon, Iran.

Background: Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic joint inflammation. The cause of rheumatoid arthritis is not known. This cause is related to a combination of hormonal or environmental and hereditary or genetic factors. One out of every hundred people will be affected by this disease in some way during their lifetime. Joint rheumatism, especially knee rheumatism, has a higher prevalence rate in women, and women suffer from this disease three times more than men. The symptoms of the disease include dryness, pain in the joints, weight loss, fever, fatigue, and weakness in different people. The aim of this study is to investigate the relationship between serum levels of ANA, Anti CCP, RF, and ESR factors in patients with suspected rheumatoid arthritis.

Methods: In this descriptive-cross-sectional study, 296 patients suspected of RA who were referred to the laboratories of Tonekabon City from April 1 to December 10, 2022, were investigated. ANA and Anti CCP on patients' samples were evaluated using the ELISA method. RF was detected quantitatively by the latex agglutination method. Results were analyzed by student T-test using SPSS software.

Results: Among the 296 suspected rheumatoid arthritis patients, 226 (76%) were women and 70 (24%) were men, and most of the studied patients were between 50 and 70 years old. Among the 254 people whose ANA was measured, 5 people were positive. ESR was positive in 66 out of 243 people. RF test was positive in 63 cases out of 255 people. Anti-CCP was also positive in 14 out of 250 people.

Conclusion: The results have shown that most of the suspected RA patients are negative in terms of ESR, ANA, Anti CCP, and RF tests, and the relationship between RA symptoms and serology tests is more significant in older people than in younger people.

Keywords: _Rheumatoid arthritis, ANA, ESR, RF, Anti CCP_

Investigation of TNF alpha serum levels in patients with bladder cancer

Mohammad Reza Haghshenas[1], Soolmaz Khansalar[1], Behrouz Jaba[1], Mohammad Javad Fattahi[1]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Bladder cancer is the most common urinary tract cancer and the second leading cause of death from genital urinary tract cancers. TNF alpha, or tumor necrosis factor-alpha, is a pro-inflammatory cytokine that is produced in a variety of cells. Including hematopoietic, non-hematopoietic, and malignant cells, TNF alpha is involved in cell proliferation, apoptosis, acute/chronic Inflammation, autoimmune diseases, and cancer. Considering the role of TNF alpha in cancer, we aimed to evaluate the serum level of this cytokine in patients with bladder cancer in comparison with the control group.

Methods: This study was performed on 90 patients (60 patients with bladder cancer and 30 healthy individuals as the control group who were included in the study according to the inclusion and exclusion criteria). After recording the demographic Inflammation. TNF alpha serum levels were measured using the ELISA method. The association between TNF alpha serum level with clinical and pathological criteria of the patient was also investigated.

Results: The results showed that there were no significant differences in TNF-a serum levels between patients with bladder cancer and the control group. The result also indicated that TNF alpha serum levels were not significantly associated with age, gender, stage, tumor grade, and invasions.

Conclusion: Our study showed that TNF alpha serum levels may not be associated with bladder cancer or tumor progression in this cancer. More studies are needed to determine the exact role of this cytokine in bladder cancer.

Keywords: Keywords: Bladder cancer, TNF alpha, Serum level

Laboratory identification of horses suspected of glanders using complementation fixation test, malleination and PCR tests

Shojaat Dashtipour[1]

1. Faculty of Paraveterinary Medicine, Bu-ali Sina University, Hamadan, Iran.

Background: The Middle East, known to be the birthplace of animal domestication, is among the few remaining geographical theaters in the world where glanders-stricken solipedes and human cases are still reported. In the Persian environment with some 60 yrs of alienation test history in its horse, mule, and donkey populations, mini or midi outbreaks of glanders are seen now and then mostly in the Western and central regions. This work aimed to characterize the phenotypic and genotypic properties of the most recently-collected isolates of Burkholderia mallei in Iran.

Methods: Bacterial culture, Biochemical tests, CFT and Flip 407-PCR, Bim A-PCR, and also Strauss reaction tests were included in the assessment.

Results: The results obtained from experiments conducted on four isolates from Tehran, Kordan, Oshnavieh, and Semirum outbreaks, displayed the expected characteristics of B. mallei.

Conclusion: Further studies are needed to improve the current knowledge of glanders in Iran.

Keywords: Glanders, Solipedes, Iran

Lack of adverse effects of cold physical plasma-treated blood from leukemia patients: a proof-of-concept study

Mina Alimohammadi[1], Monireh Golpour[2], Alireza Rafiei[۲]

1. Shahid Beheshti University of medical sciences, Tehran, Iran.
2. Mazandaran University of Medical Sciences, Tehran, Iran.

Background: Chronic lymphocytic leukemia (CLL) is the most common blood malignancy with multiple therapeutic challenges. In recent years, cold atmospheric plasma (CAP) has been considered a promising approach in cancer therapy.

Methods: In this study, we aimed to evaluate the cytotoxic effect of CAP or CAM on hematologic parameters in the whole blood of CLL patients.

Results: The mean RBC, WBC, platelet and hemoglobin counts, and peripheral blood smear images did not show a significant difference between treated and untreated samples in both CLL and healthy individuals. However, both direct CAP and indirect CAM treatment increased lipid peroxidation and nitric oxide production in the whole blood of CLL patients and healthy subjects. In addition, the metabolic activity of WBCs was decreased with 120 seconds of CAP or CAM treatment after 24 hours and 48 hours. But CAP and CAM treatment did not affect the PT/PTT and hemolysis in both CLL patients and healthy individuals. The present study identifies the components of CAP to reach the blood without disturbing the basic parameters of hematology, confirming the idea that the effect of CAP may not be limited to solid tumors but may extend to hematological disorders.

Conclusion: Further cellular and molecular studies are needed to determine precisely which cells in CLL patients are targeted by CAP or CAM.

Keywords: Chronic lymphocytic leukemia (CLL), whole blood, hematologic parameters, adverse effects, plasma medicine

Sperm Antibodies and Male Infertility

Omid Moeini[1], Seyed Armit Hosseini[1], Mohammad Matin Nourikhani[1], Amir Gholamzad[1], Mehrdad Gholamzad[2]

1. Department of Laboratory Medicine, Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
2. Department of Microbiology and Immunology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.

Background: Male infertility is a complex disease and can be caused by different factors, including immune factors such as the presence of sperm antibodies. However, limited research has examined the relationship between sperm antibody presence and impaired sperm function.

Methods: Sperm samples were collected from 100 patients experiencing reproductive problems, and the presence of sperm antibodies was examined using a mixed antiglobulin reaction assay.

Results: 57% of the patients were found to have sperm antibodies, with 30% having high titers of antibodies. The presence of sperm antibodies was associated with decreased sperm motility, abnormal sperm morphology, and poor sperm function.

Conclusion: The study results suggest that the presence of sperm antibodies is a potential cause of male infertility and may cause impaired sperm function. Therefore, it is important to consider the role of sperm antibodies in infertility diagnosis and management. Further research is needed to explore the effectiveness of new therapeutic strategies targeting sperm antibodies in the treatment of male infertility

Keywords: Infertility, Cytokine, Reproductive Biology

The Accuracy of CA125, HE4, and ROMA in Ovarian Cancer Diagnosis among Premenopausal and Postmenopausal Women with Pelvic Mass

Dorsa Alijanzadeh[1], Saba Mardekatani Asl[1], Zahra Mohammadi[1], Sedighe Vafaei[2], Zahra Shiravani[3], Mohammad Javad Fattahi[4], Mahdi Shabani[5]

1. Student Research Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Synapse IVD accelerator, Tehran, Iran
3. Gynecology Oncology Division, Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
5. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: To evaluate the diagnostic values of cancer antigen 125 (CA125), human epididymis protein 4 (HE4), and risk of malignancy algorithm (ROMA) and their differentiation power between benign and malignant ovarian tumors.

Methods: In this observational study, the CA125 and HE4 serum levels were measured using the ELISA technique in 72 patients before undergoing surgery for pelvic mass from March 2018 to October 2020. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of CA125, HE4, and ROMA were calculated and receiver operating characteristics (ROC) and areas under the curves (AUC) were checked for further analysis.

Results: There were 45 (62.5%) women with benign ovarian tumors and 27 (37.5%) patients with malignant ovarian cancer. The serum level of CA125 and HE4 of patients with malignant tumors were significantly higher than in the patients with benign pelvic mass ($p<0.001$ for both biomarkers). The calculated ROMA also was significantly higher among patients with ovarian cancer ($p<0.001$). Furthermore, ROMA had the highest sensitivity (85.2%) and NPV (90.0%); and HE4 had the best specificity (91.1%) and PPV (81.8%). In the pre-menopause subgroup, ROMA had a good performance with a sensitivity of 77.8% and specificity of 84.6%. In the post-menopause subgroup, HE4 and ROMA had 100% sensitivity, but HE4 had better specificity compared to the ROMA (84.2% vs. 73.7%).

Conclusion: In this study, we emphasized the promising role of HE4 for better detection of ovarian cancer. In the pre-menopause subgroup, HE4 would be the best indication for detecting malignant cases. In the post-menopause subgroup, HE4 and ROMA proved to be the best biomarker indications.

Keywords: Ovarian Cancer, CA125, HE4, ROMA

The effects of cold atmospheric plasma (CAP) on the function of exhausted CD8+ T cells in patients with chronic lymphocytic leukemia

Mina Alimohammadi[1], Monireh Golpour[2], Alireza Rafiei[۲]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Mazandaran University of Medical Sciences, Mazandaran, Iran.

Background: Cold atmospheric plasma (CAP) treatment has yielded invaluable success in tumor therapy as well as remarkable clinical responses in a wide range of cancers. Patients with chronic lymphocytic leukemia (CLL) continue to relapse and progress despite common treatment protocols that have improved overall survival.

Methods: The current in vitro study investigated the use of CAP to restore the function of exhausted CD8+ T cells in CLL. CD8+ T cells were isolated from the peripheral blood of 24 patients with CLL, treated with CAP, and cocultured with mitomycin-frozen CD19+ B cells as target cells. Cultures were stimulated with anti-CD3/CD28 antibodies to assess the proliferation of CD8+ T cells by MTT and stimulated with PMA/ionomycin to measure the expression of CD107a, PD1, and CTLA-4 and cytokine production by flow cytometry and ELISA, respectively.

Results: Our results showed that CAP therapy improved the proliferation of CD8+ T cells in CLL patients. Representative flow cytometry data from CLL patients and untreated subjects revealed that CD107a expression on stimulated CD8+ T cells from CLL patients cultured without target cells was higher than that of untreated controls, indicating that their degranulation activities were enhanced. In addition, the percentage of PD1+ and PD1+CTLA-4+ cells were comparable between the CAP-treated and untreated groups, with no significant difference observed. However, the percentage of CTLA-4+ cells in the CAP-treated group was significantly lower than in the untreated group. Real-Time PCR also demonstrated that CAP treatment had no effect on the gene expression of PD1 and CTLA-4 in both CLL patients and untreated subjects there was a significant difference between the control and treated groups in terms of degranulation properties and CD8+ T cell production of IFN-, TNF-, and IL-10.

Conclusion: More in vitro and in vivo studies are needed to investigate the utility of CAP treatment for CLL patients.

Keywords: cold atmospheric plasma (CAP), chronic lymphocytic leukemia, T-CD8+ cells, CD107a, PD1, CTLA-4, inflammatory cytokines

COVID-19 Immunology and Vaccines

Evaluation of MDA-5 and IFN-βgenes expression in the patients with COVID-19

Parisa Feizollahi[1], Sara Falahi[1], Ali Gorgin Karaji[1]

1. Department of Immunology, School of Medicine, Kermanshah University of Medical, Sciences, Kermanshah, Iran.

Background: Emerged coronavirus disease 2019 (COVID-19) is a pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2). The innate immune system functions as the first and main line of host defense against viral infections, such as SARS-CoV-2. Pattern recognition receptors of the innate immune system, such as retinoic acid-inducible Gene-I (RIG-)-like receptors (RLR), including RIG-I (retinoic acid-inducible gene I) and MDA5, are responsible for recognizing viruses and inducing Interferon production. IFN production pathways are considered as a substantiate arm of the innate immune system against viral infection and RIG-I and MDA5 are the most well-studied RLRs. Considering the contribution of RLR signaling in immune-mediated reactions, this study was conducted to investigate the association between gene expression of MDA-5 and IFN-βin patients with COVID-19.

Methods: Forty patients with severe COVID-19 and 40 patients with a mild form of COVID-19 were enrolled in this study. Blood samples were obtained from healthy controls and patients. After RNA extraction, the RT-qPCR technique was used to evaluate the expression level of the studied genes.

Results: Data showed that the expression level of MDA-5 and IFN-βwas significantly higher in the mild patients group than in the patients with the severe form of COVID-19. (P=0.021, P=0.032 respectively).

Conclusion: Our results pointed to the role of MDA-5 and IFN-β against SARS-CoV-2 and decreased levels of them may be associated with COVID-19 severity.

Keywords: MDA-5, IFN-β SARS-CoV-2, COVID-19

A Bioinformatics Investigation on Neutrophils' Role in Coronavirus Infection: Are They Beneficial or Detrimental Immune Cells?

Nima Hemmat[1], Zahra Asadzadeh[1], Behzad Baradaran[1]

1. Tabriz University of Medical Sciences, Tabriz, Iran.

Background: The Coronavirus Disease 2019 (COVID-19) caused by the SARS-CoV-2 virus has become a pandemic and a serious global health threat. This new virus is capable of infecting lower respiratory tract cells, causing severe acute respiratory syndrome, pneumonia, and even death. The underlying molecular mechanisms of COVID-19 are not yet fully understood.

Methods: In this study, we analyzed the GSE1739 microarray dataset containing 10 SARS-positive PBMCs and four normal PBMC. Through co-expression network analysis using the WGCNA package of R v3.0, we identified the 833-turquoise module with genes significantly associated with SARS-CoV infection.

Results: we found that genes such as ELANE, ORM2, RETN, BPI, ARG1, DEFA4, CXCL1, and CAMP were crucially involved in the disease, based on GEO2R analysis. The GO analysis showed that the biological processes most activated in SARS-CoV infection are neutrophil activation and neutrophil degranulation, as well as the predicted neutrophilia, basophilia, and lymphopenia by deconvolution analysis of samples.

Conclusion: the use of Serpins and Arginase inhibitors could potentially increase the survival rate of SARS-positive patients. Considering the high similarity between SARS-CoV-2 and SARS-CoV, these inhibitors could also be beneficial for COVID-19 patients.

Keywords: Bioinformatics, Coronavirus Disease 2019, Micro-array, Neutrophils

A large retrospective Cohort study of laboratory symptoms of COVID-19 disease in various comorbidities

Mahdieh Shokrollahi Braough[1,2], Masoud Yunesian[3], Mohammad Darzi[4], Danesh Amini Panah[5], Samaneh, Dehghani[6], Yekta Ghane[7], Sam Mottahedan[2], Sohrab Sakinehpour[8], Tahereh Kowsarirad[8], Zahra Hosseini-Farjam[2], Mohammad Reza Amirzargar[shokri], Fahimeh Shahriyary[9], Mohammad Mahdi Kabiri[2], Zeynab Yassin[10], Nazanin Mojtabavi[1]

1. Department of Immunology, Iran University of Medical Sciences, Tehran, Iran
2. ATMP Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
3. Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4. Genetics Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
5. Antimicrobial resistance research center, institute of immunology and infectious diseases Iran University of Medical Sciences, Tehran, Iran
6. Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
7. School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
8. Radiation Sciences Department, School of paramedicine, Iran University of Medical Sciences, Tehran, Iran
9. Department of Hematology & Blood Banking, School of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
10. Department of Infectious Disease, School of Medicine, Antimicrobial Resistance Research Center, Iran University of Medical Sciences, Tehran, Iran

Background: COVID-19 is one of the most critical respiratory viral pandemics that has resulted in many deaths. Different variants of the virus have created many infection peaks throughout the world as a result of the virus' many genetic mutations. The clinical symptoms and laboratory parameters can be essential in determining prognosis and treatment options. This study investigated comorbidities and laboratory parameters in determining the severity and prognosis of the disease in different peaks of the virus.

Methods: This retrospective cohort study was conducted on 7,500 patients with Covid-19 admitted to Hazrat Rasool Akram Hospital between 2019 and 2022. The COVID-19 viral peaks were defined according to the Pastur institute data. The hospital data of 50 parameters, including nine comorbidities, five general information, and 35 laboratory parameters, were collected from the HIS system, then sorted by advanced coding of MATLAB software, and then statistically analyzed in SPSS and R software.

Results: The serum level of inflammatory enzymes such as CRP, BUN, ALP, SGOT, and SGPT and the ratio of lymphocytes to total leukocytes varied in different comorbidities. In diabetes and kidney failure, the risk of death and length of hospitalization could be predicted by laboratory parameters with high accuracy over 80%. The COVID-19 severity prediction showed an age-dependent spectrum in ages over 60 and young patients; the mortality rate was virus-strain dependent. The risk of death in patients with comorbidities was more than 20 times that of healthy patients.

Conclusion: Investigation of the inflammatory parameters and comorbidities revealed that patients without comorbidities have a higher survival rate with lower inflammatory parameters. The delta virus-related hospitalization was more than other strains, and o-micron-related disease was highly fatal in older patients compared to younger ones.

Keywords: COVID-19, laboratory data, Comorbidity, MATLAB-Coding

A nested PCR method to track down COVID-19 spike gene sequence

Soheila Golchin[1], Reza Valadan[2]

1. MSc in Immunology, Gastrointestinal Cancer Research, Non-communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Immunology, Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: Coronavirus genome sequencing is essential for understanding the spread and control of the virus. It is important to keep track of new variants because the vaccine must be updated to match current virus strains. The spike gene plays a critical role in covid-19 pathogenesis and identification of polymorphism in this gene can be helpful for controlling and detecting variants that can evade vaccine immunity.

Methods: This study was conducted on patient samples before and after the outbreak of the coronavirus. Nasal and Pharyngeal samples were taken from covid positive patients and the presence of the virus was detected using qualitative RT-PCR. Overlapping primers were designed to amplify the entire spike gene in a nested PCR assay.

Results: In This assay using a conserved whole sequence of spike genes was amplified and sequenced using 3 pairs of conserved overlapping primers. It could accurately differentiate delta and omicron variants in patients. It could also detect critical mutations in the sequence of spike RBD that may provide a base for the immune escape mechanism.

Conclusion: This method can detect the strains that will arise in the future and is useful in monitoring the virus and making vaccines specific to the strain.

Keywords: Covid-19, Gene sequencing, nested PCR, Polymorphism

A Systematic Review of Pleiotropic Potential of Erythropoietin as an Adjunctive Therapy for COVID-19

Fatemeh Emamipari[1], Soroush Kamali[2], Ghazaleh Nikzad[3], Narges Roosta Navi[4], Sanaz Soltani[5], Mohamad Reza Kalani[6]

1. Department of Animal Sciences, College of Life Sciences, Kharazmi University of Tehran, Tehran, Iran.
2. Department of Molecular Cell Biology - Molecular Cell Science, Faculty of Basic Sciences, Mohaghegh Ardabili University, Ardabil, Iran.
3. Department of Molecular Cell Biology - Molecular Cell Science, Faculty of Basic Sciences, Mohaghegh Ardabili University, Ardabil, Iran.
4. Department of Molecular Cell Biology - Molecular Cell Science, Payame Noor University of Talesh, Gilan, Iran.
5. Department of Biology, Shahr-e-Quds Branch, Islamic Azad University, Tehran, Iran.
6. Molecular Medicine Department, Golestan University of Medical Sciences, Gorgan, Iran.

Background: Coronavirus disease 2019 (COVID-19) is a severe acute respiratory disease with a high prevalence. According to the research and statistical data, in January 2021, there have been 92,262,621 confirmed cases of COVID-19 and more than two million deaths. Infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the main cause of this disease. In addition to the respiratory system, the disease affects the gastrointestinal tract, central-peripheral nervous system, circulatory system, and kidneys. Therefore, any therapeutic action to reduce COVID-19-related symptoms and complications is essential.

Methods: In this study, we conducted a systematic review of the published literature and preprints on the efficacy of erythropoietin (EPO) and recombinant human EPO as a safe stimulant and tissue protector in the treatment of COVID-19. We also briefly described the structure of coronavirus, its pathogenesis, and the structure of EPO and recombinant human EPO. All relevant articles published in the Science Direct, PubMed, and Google Scholar databases were searched.

Results: According to the results, EPO is a cytoprotective cytokine induced by hypoxia. The pleiotropic effects of EPO are associated with its erythrocyte-forming, anti-apoptotic, anti-inflammatory activities. It also exerts protective effects on the heart, lungs, kidneys, arteries, and central and peripheral nervous systems. It has been demonstrated that EPO can increase hemoglobin levels, thereby increasing oxygen delivery to the tissues.

Conclusion: Therefore, recombinant human EPO therapy can be used for counteracting the adverse effects of COVID-19 including hypoxic myocarditis, acute renal failure, pulmonary edema, and brain-spinal cord ischemic injury. Overall, the use of EPO and recombinant human EPO therapy increases blood coagulation, tumor growth, thromboembolism, and purification of red blood cells, which must be accompanied by anticoagulants such as heparin.

Keywords: Erythropoietin, COVID-19, Hypoxia, Recombinant human erythropoietin, Pleiotropic

Adverse events of special interest after vaccination with Covid-19 vaccines: A Cohort Study

Roqayeh Aliyari[1], Sepideh Mahdavi[1], Mostafa Enayatrad[2], Sajad Sahab-Negah[3], Sairan Nili[4], Mohammad Fereidooni[5], Parvin Mangolian Shahrbabaki[6], Alireza Ansari-Moghadam[7], Abtin Heidarzadeh[8], Fariba Shahraki-Sanavi[9], Mahlagha Dehghan[10], Mohammadreza Amini Moridani[11], Mansooreh Fateh[12], Hamidreza Khajeha[14], Zahra Emamian[14], Elahe Behmanesh[15], Hamid Sharifi[15], Mohammad Hassan Emamian[13]

1. Department of Epidemiology, School of Public Health, Shahroud University of Medical Sciences, Shahroud, Iran.
2. Clinical Research Development Unit, Bahar Hospital, Shahroud University of Medical Science, Shahroud, Iran
3. Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Department of Public Health, Faculty of Health, Kurdistan University of Medical Sciences, Sanandaj, Iran
5. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.
6. Nursing Research Center, Razi Faculty of Nursing and Midwifery, Department of Critical Care, Kerman University of Medical Sciences
7. Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.
8. School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
9. Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.
10. Nursing Research Center, Razi Faculty of Nursing and Midwifery, Department of Critical Care, Kerman University of Medical Sciences
11. Health deputy, Guilan University of Medical Sciences, Rasht, Iran.
12. Center for Health Related Social and Behavioral Sciences Research, Shahroud University of Medical Sciences, Shahroud, Iran.
13. Ophthalmic Epidemiology Research Center, Shahroud University of Medical Sciences, Shahroud, Iran.
14. Health Technology Incubator Center, Shahroud University of Medical Sciences, Shahroud, Iran.
15. HIV/STI Surveillance Research Center, and WHO Collaborating Center for HIV Surveillance, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, Iran.

Background: To determine the incidence of adverse events of special interest (AESIs) among Iranians who are vaccinated with Covid-19 vaccines.

Methods: This is a cohort event monitoring (CEM) study for safety signal detection after vaccination with COVID-19 vaccines based on a guideline, provided by World Health Organization. The participants were in the seven cities of Iran that have received Sinopharm, Sputnik V, AZD1222, and COVIran Barekat vaccines according to the national instructions. They were followed up for 17 weeks (25 weeks for AZD1222) after receiving the first dose of vaccine by weekly phone contacts or self-reporting in a web-application. All hospitalized cases were investigated for causes of admission by a classification committee.

Results: Thirteen cases of AESIs were recorded among the 31690, 20195, 23780, and 14118 participants who were vaccinated with Sinopharm, Sputnik V, AZD1222, and COVIran Barekat respectively. The AESIs were identified among 2080 hospitalized cases and included five cases of coagulation disorders, three after AZD1222 and two after Sinopharm vaccination, as well as four cases of generalized convulsion after receiving Sinopharm, Sputnik, and AZD1222 (two cases) vaccines. In addition, four cases of Guillain-Barre syndrome, Diabetic ketoacidosis, Pericarditis, and Sub-Acute Thyroiditis were detected after receiving Sputnik V, Sinopharm, COVIran Barekat, and Sputnik V respectively.

Conclusion: The incidence of AESIs after Covid-19 vaccines is relatively rare and the studied vaccines can be considered safe. AZD1222 had a higher incidence of AESIs. It should be noted that the number of participants was not similar between vaccine brands and the comparison of vaccines for the incidence of AESIs should be done with caution.

Keywords: Adverse events; Covid-19; Vaccine; Iran

An analysis of vitamin D serum levels in rats post-immunized with inactivated feline coronavirus (FCoV) and COVID-19 viruses

Mahmood Ahmadi Hamedani[1], Mehrshid Mehravaran[1], Khatereh Kafshdouzan[2], Hamid Staji[3], Mohammad Hasan Yousefi[4]

1. Department of Clinical Sciences, School of Veterinary Medicine, Semnan University, Semnan, Iran
2. Department of Pathobiology, School of Veterinary Medicine, Semnan University, Semnan, Iran
3. Department of Pathobiology, School of Veterinary Medicine, Semnan University, Semnan, Iran
4. Department of Basic Sciences, School of Veterinary Medicine, Semnan University, Semnan, Iran

Background: Since there is no data on serum vitamin D levels in cats infected with feline coronavirus (FCOV) and humans after infection with the COVID-19 virus or after vaccination with inactivated COVID-19 virus, is the continued use of vitamin D supplements how necessary? This study aimed to investigate the serum vitamin D levels in Wistar rats receiving two doses of inactivated FCOV and COVID-19 after 35 days.

Methods: Thirty healthy adult male Wistar rats were randomly assigned to three experimental groups of ten, including the control group (Group I) and the virus-receiving groups (Group II and III). Rats of groups I, II, and III were administered 0.5 ml of hydroxide aluminum (HA) and one dose (107 Tissue culture infectious dose 50% (TCID50) of FCOV and COVID-19 viruses subcutaneously in the back of the neck, respectively. HA and booster were administered to the control group and the virus-receiving groups three weeks after the first injection. Blood samples were obtained from the rats of each group before the beginning experiment and 14 days after the second administration (day 35), and then samples were stored frozen until analysis.

Results: The serum levels of vitamin D were not significantly different between group I (59.05 ± 7.67), group II (58.93 ± 7.35), and group III rats (59.25 ± 7.15) on day 0, while this vitamin in group II and III rats showed a significant decrease (46.82 ± 5.84 and 50.46 ± 4.82; $P < 0.05$) compared to group I (60.19 ± 7.33) on day 35.

Conclusion: Our data showed serum vitamin D levels decreased in rats post-immunized with inactivated FCoV and COVID-19 viruses. There appears to be a need to continue using vitamin D in cats with feline infectious peritonitis virus and humans vaccinated with covid-19.

Keywords: feline coronavirus, COVID-19, Vitamin D.

Anti-SARS-CoV-2 spike IgG following injection of the third dose vaccine: A systematic review with meta-analysis of heterologous versus homologous vaccination

Mohammad-Shafi Mojadadi[1], Seyed Alireza Javadinia[2], Fahimeh Attarian[3], Elham Samami Samami[4], Imona Sobhani[5]

1. Department of Immunology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.
2. Non-Communicable Diseases Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran.
3. Department of Epidemiology and Biostatistics, School of Health, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran.
4. University of Florida Health Cancer Center, Gainesville, FL, United States
5. Student Research committee, Sabzevar University of Medical Sciences, Sabzevar, Iran.

Background: Mass vaccination is a key strategy to prevent and control the coronavirus disease 2019 (COVID-19) pandemic. Today, several different types of vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been developed worldwide. These vaccines are usually administered in a two-dose schedule, and the third dose is currently being administered in most countries. This study aimed to systematically review and Meta-analyze the immunogenicity of heterologous versus homologous vaccination after the administration of the third dose of COVID-19 vaccines.

Methods: Electronic databases and websites including Scopus, PubMed, Web of Science, and Google Scholar were searched for relevant randomized clinical trial (RCT) studies. After applying the inclusion and exclusion criteria, a total of three RCTs were included in the study. These RCTs included 2613 healthy adults (18 years or older and without a history of laboratory-confirmed COVID-19) with 15 heterologous and five homologous prime-boost vaccination regimens. Anti-SARS-CoV-2-spike IgG levels at day 28 after administration of the third dose, were compared between the heterologous and homologous regimens.

Results: The highest antibody responses had been reported for the homologous vaccination regimen of m1273/m1273/m1273 (Moderna), followed by the heterologous regimen of BNT/BNT/m1273. In addition, the immunogenicity of viral vectors and inactivated vaccines was remarkably enhanced when they had been boosted by a heterologous vaccine, especially mRNA vaccines.

Conclusion: This systematic review suggests that mRNA vaccines in a homologous regimen induce strong antibody responses to SARS-CoV-2 compared to other vaccine platforms. In contrast, viral vector and inactivated vaccines show satisfactory immunogenicity in a heterologous regimen, especially in combination with mRNA vaccines.

Keywords: SARS-CoV-2, COVID-19 vaccine third dose, Heterologous vaccination, Homologous vaccination, Anti-SARS-CoV-2 antibod

Ascorbic acid and αtocopherol in the inactivated SARS-CoV-2 vaccine formulation: A comparison study in aged and young mice

Nika Asefi[1], Parviz Pakzad[2], Akbar Khorasani[3], Morteza Taghizadeh[4], Mohammad Hossein Yazdi[5], Mehdi Mahdavi[6]

1. Department of Microbiology, Faculty of Basic Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran.
2. Department of Microbiology, Faculty of Basic Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran.
3. Department of FMD vaccine production, Razi Vaccine & Serum Research Institute, Agricultural Research, Education & Extension Organization (AREEO) Karaj, Iran.
4. Department of Human Vaccine, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.
5. Biotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
6. Advanced Therapy Medicinal Product (ATMP) Department, Breast Cancer Research Center, Motamed Cancer Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran.

Background: The function of the immune system gradually declines over the lifespan of humans. Aging causes myriad biological changes in the immune system, such as immune defenses and susceptibility to infectious diseases. This leads to a decrease in the ability to defend against antigens and the survival rate. With the advent of coronaviruses in early December 2020, morbidity and mortality have mainly been observed in elderly populations. Therefore, finding an appropriate treatment to increase immune function in these patients is essential. In the present study, we compared the effect of the combination of ascorbic acid and αtocopherol in the formulation of inactivated SARS-CoV-2 vaccine on aged and young mice population.

Methods: A SARS-CoV-2 strain was isolated from the patient and then cultured in the Vero cell line. The isolated and propagated virus was inactivated using formalin and purified using column chromatography. Inactivated SARS-CoV-2 was formulated in Alum adjuvant combined with Vit-C or αtocopherol and /or both of them. The vaccines were injected twice into young and aged C57BL/6 mice. Two weeks later, IFN-γ, IL-4, and IL-2 cytokines were assessed using ELISA kits. Specific IgG and IgG1/IgG2a were assessed by an in-house ELISA.

Results: The results of IL-4 and IFN-γ cytokines showed a significant increase in both the aged and young mice population versus the Alum-based vaccine. In addition, a significant decrease in specific total IgG was observed, while IgG2a/IgG1 ratio was increased.

Conclusion: The results showed that using ascorbic acid and alpha-tocopherol simultaneously in the formulation of inactivated SARS-CoV-2 vaccine has a potential impact on the Th1 immune response in the aged mice population.

Keywords: Inactivated SARS-CoV-2 vaccine, elderly, Ascorbic acid, Alpha-tocopherol, Adjuvant

Association between angiotensin-converting enzyme-2 gene polymorphism (rs2108609) with severity and outcome of COVID-19 infection

Hamideh Mohammadi-Berenjestanaki[1], Elaheh Mohammadali[2], Mina Khasayesi[3], Alireza Rafiei[4], Zahra Kashi[5], Narges Mirzaei Ilali[6], Zahra Hosseni-Khah[7]

1. M.Sc. of Microbiology, Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran
2. M.Sc. of Genetic, Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran
3. M.Sc. of Biochemistry, Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran
4. Professor of Immunology, Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
5. Professor of Endocrinology, Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran
6. Internal Medicine Resident, Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran,
7. Molecular Medicine, Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran

Background: COVID-19 which is caused by SARS-CoV-2 affected hundreds of millions of people and it still has a seemingly endless stream. Since SARS-CoV-2 binds to the ACE2 receptor on the surface of host cells through the spike proteins, ACE2 rs2106809 polymorphism may be related to the risk and severity of COVID-19. This study aimed to investigate the association between ACE2 rs2106809 polymorphism as well as the influence of this polymorphism on disease severity in patients with COVID-19.

Methods: In this cross-sectional study, ACE2 rs2106809 polymorphism was assessed in 142 patients (45 severe and 97 non-severe forms) with SARS-CoV-2 infection admitted to the teaching hospitals of Mazandaran University of Medical Sciences in March-April and June-July 2020. Genomic DNA was extracted from the whole blood of the patients using a commercial genomic extraction kit. Polymerase Chain Reaction (PCR)–based restriction fragment-length polymorphism (RFLP) was performed to genotype the ACE2-rs2108609 with specific primers and Taq1 restriction enzyme.

Results: The results showed that G/G genotype was significantly associated with COVID-19 severity in all patients (p=0.0007). Analysis showed that ACE2 rs2106809 was associated with the disease outcome (p <0.05) in all patients.

Conclusion: G/G genotype of ACE2 rs2106809 was associated with the severity and clinical outcome of patients with COVID-19.

Keywords: ACE2 rs2108609 polymorphism, COVID-19 infection, Angiotensin-converting enzyme (ACE) inhibitors, Severity

Association between expression of TLR 3, 7, and 8 genes and severity of COVID-19

Nasir Arefinia[1], Amir Sasan Mozafarinejad[2]

1. Jiroft University of Medical Sciences, Jiroft, Iran
2. Jiroft University of Medical Sciences, Jiroft, Iran

Background: Innate immunity responses following the sensing of the virus signature by cellular sensors including TLRs play a vital role in the pathogenesis and outcome of viral diseases including COVID-19. In this study, we aimed to investigate the expression levels of TLR3, TLR7, and TLR8 in COVID-19 patients and their correlation with disease severity and outcome.

Methods: 75 quantitative Real-Time PCR (qRT-PCR)-confirmed COVID-19 patients were included consecutively and divided into 3 groups of mild, severe, and critical based on the severity of the disease. Also, 25 age and gender-matched healthy volunteer subjects were included. PBMCs were collected from the whole blood, and RNA was extracted using a commercial kit. The expression of TLR3, TLR7, and TLR8 genes was investigated using the qRT-PCR technique.

Result: The mean age of the patients and healthy volunteers was 52.69±1.9 and 49.12±2.7, respectively. In each group, 13 out of 25 participants were male. The expression levels of TLR3 (p <0.001), TLR7 (p <0.001), and TLR8 (p <0.001) transcript were significantly higher in COVID-19 patients than in the control group. The results also revealed that the expression levels of TLR7 and TLR8 were significantly higher in the critical and severe COVID-19 patients compared to those with mild disease (p<0.05). In addition, the result showed a significant elevate in TLR3 transcript in critical compared to mild patients (p=0.01). Moreover, regarding gender, the expression levels of TLR8 were significantly elevated in the male severe (p=0.02) and critical (p=0.008) patients than the female ones. TLR3 (p=0.2) and TLR7 (p=0.08) transcripts were elevated in males than female but not significantly.

Conclusion: The results indicated that TLR3, TLR7, and TLR8 genes might have an important role in the severity of COVID-19 disease. Moreover, the severity of COVID-19 disease in male patients might be related to TLR8 expression levels. More studies are recommended to verify this issue.

Keywords: TLR3, TLR8, TLR7, COVID-19, SARS-CoV2

ASSOCIATION BETWEEN EXPRESSION OF ZBP1, AIM2, AND MDA5 GENES AND SEVERITY OF COVID-19

Nasir Arefinia[1], Amir Saan Mozafari[1]

1. Jiroft University of Medical Sciences, Jiroft, Iran.

Background: Antiviral and inflammatory responses following the detection of the virus genome by nucleic acid sensors play a vital role in the pathogenesis and outcome of diseases. In this study, we investigated the ZBP1, AIM2, and MDA5 expression levels in COVID-19 patients with different intensities of the disease.

Methods: Seventy-five quantitative Real-Time PCR (qRT-PCR)-confirmed COVID-19 patients were included consecutively and divided into 3 groups mild, severe, and critical based on the severity of the disease. Also, 25 healthy volunteer subjects were included. PBMCs were collected from the whole blood, and RNA was extracted using a commercial kit. The expression of ZBP1, AIM2, and MDA5 genes was investigated using the qRT-PCR technique.

Results: The mean age of the patients and healthy volunteers was 52.73±13.78 and 49.120±12.490, respectively. In each group, 13 out of 25 participants were male. The expression levels of ZBP1 (p=0.001), AIM2 (p=0.001), and MDA5 (p =0.003) transcript were significantly higher in COVID-19 patients than in the control group. The results also revealed that the expression levels of ZBP1, AIM2, and MDA5 were significantly higher in the critical and severe COVID-19 patients compared to those with mild disease (p<0.05). Moreover, regarding gender, the expression levels of AIM2 and MDA5 were significantly elevated in male severe (p=0.04 and p=0.003, respectively) and critical (p=0.005 and p=0.0004, respectively) patients than the female ones.

Conclusion: The results indicated that ZBP1, AIM2, and MDA5 genes might have an important role in the severity of COVID-19 disease. Moreover, the severity of COVID-19 disease in male and female patients might be related to AIM2, and MDA5 expression levels. More studies are recommended to be conducted to clarify this issue.

Keywords: ZBP1, AIM2, MDA5, SARS-CoV-2, COVID-19, severity of COVID-19

Association between hematological and biochemical markers and disease prognosis in COVID-19.

Minoo Shahidi[1] *; Fahimeh Shahriary[1] ; Fatemeh Sadat Hosseini-Baharanchi[2] ; Fatemeh Shahabi[2] ; Payamsiadat[1] ; Ali Sadeghi[3] ; Samira Bagheri[3] ; Hanieh Beiranvand[3] ; Bahareh Moghimian[3,4]

1.Department of Hematology and Blood Banking, School ofAllied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran.
2. Department of Hematology, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran.
3. Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran.
4. Shahid Beheshti Hospital, Ardestan, Isfahan, Iran.

Background: Many studies have pointed out the role of laboratory tests in the diagnosis of COVID-19, however, hematological and biochemical markers in the prognosis have not yet been definitely established. We aimed to investigate a possible association between these markers and the prognosis of the disease.

Methods: Demographic, clinical laboratory and outcome data were extracted from available medical records of 60 confirmed COVID-19 patients (31 males, 29 females; mean age64.43±17.52) were extracted (available medical records). Patients were assigned to two groups: a "recuperated/recovered" group and a "deceased" group. Blood examinations involved complete blood count and serum biochemical factors such as Creatinine (Cr), Urea, Sodium (Na), and Potassium (K). Inflammatory indicators, including C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), and erythrocyte sedimentation rate (ESR) were assessed in terms of age and gender in both groups. The parameters were assessed again at the time of discharge and compared with those at admission.

Results: Elevated neutrophil, NLR, and Sodium were significantly correlated with adverse clinical outcomes (P= 0.030, 0.037, and 0.032 respectively). Low levels of lymphocytes increased the occurrence of death in females (p= 0.011). Low platelet counts significantly increased the death rate in patients > 60 years old (p= 0.021). CRP and ESR were significantly increased in patients. Higher ages were also significantly associated with poor clinical outcomes (p=0.031).

Conclusions: Routine laboratory parameters such as NEUT, LYMP, NLR, PLT, and Na can be valuable indicators for the prediction of severity and patients' prognosis upon hospital admission as well as during therapeutic interventions. ICIA 2023 16th International Congress of Immunology and Allergy

Keywords: SARS-CoV-2, COVID-19, Blood cell count, Neutrophil lymphocyte ratio (NLR), Hematological parameters, Biochemical parameters, inflammatory markers

Association of ABO blood groups and rhesus antigen distribution with COVID-19 in Iran

Maryam Nejabat[1], Nazanin Nazari[2], Asmaalsadat Nikkhouy Tanha[3], Nasim Afshari[4], Fatemeh Rahmanzad[5], Esmaeil Rezaei[6], Mohammad Motamedifar[7], Saeid Amirizadeh Fard[8]

1. Shiraz HIV/AIDS Research Center, Institute of Health & Dept. of Bacteriology and Virology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Department of plant Protection, College of Agricultural Biotechnology Sciences, Shahed University, Tehran, Iran.
4. Department of Microbiology, Islamic Azad University Science & Research Branch, Tehran, Iran.
5. Department of immunology, College of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.
6. Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.
7. Shiraz HIV/AIDS Research Center, Institute of Health & Dept. of Bacteriology and Virology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran.
8. Diagnostic Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz, Iran.

Background: We aimed to elucidate the association between ABO blood type, Rhesus antigen, and SARS-CoV-2.

Methods: In this study, 329 patients diagnosed with COVID-19 were selected between April-July, 2021 at the Laboratory of Pathobiology and Genetics of Peyvand, Shiraz, Iran.

Results: Individuals with blood type AB were more susceptible to COVID-19 infection than non-AB blood type (p<0.0001). Rh-positive individuals are at lower risk of viral infection than Rh-negative individuals (p<0.01). A correlation was found between the ABO blood group distribution and Myalgia or fatigue (p<0.01).

Conclusion: Patients with blood group AB had an increased risk for infection with SARS-CoV-2, while Rh-positive was associated with a decreased risk, indicating that certain ABO blood groups were correlated with SARS-CoV-2 susceptibility.

Keywords: SARS-CoV-2, Abo blood group, rhesus antigen

Association of the CTLA4 gene +49A>G single-nucleotide polymorphism (rs231775) with Covid-19 severity in Iranian population

Ensie Sadat Mirsharif[1], Abdolrahman Rostamian[2], Mohammadreza Salehi[3], Nayere Askari[4], Tooba Ghazanfari[1]

1. Immunoregulation Research Center, Shahed University, Tehran, Iran
2. Rheumatology Research Center, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Infectious and Tropical Medicines, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Biology, Faculty of Basic Sciences, Shahid Bahonar, University of Kerman, Kerman, Iran.

Background: Literature evidence indicated that the +49A>G single-nucleotide polymorphism (SNP), which is in the first exon of the cytotoxic T lymphocyte-associated 4 (CTLA4) gene, is related to some immune-mediated diseases. The present hospital-based case-control study aimed to investigate the genetic association between the +49A> G SNP of the CTLA4 gene and COVID-19 severity.

Methods: This polymorphism of the CTLA4 gene was detected by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 794 COVID-19 patients and 167 control subjects.

Results: According to online SNPstats software analyses, the frequencies of this SNP were in accordance with the Hardy-Weinberg equilibrium (P=0.57) in the control group and this SNP can be used as a genetic marker to associate the CTLA4 gene and COVID-19. There were no significant differences between study groups in the frequency distribution of alleles and genotypes of the +49A>G SNP under different models of inheritance. In addition, further analysis following subdivision by gender showed that the frequency of alleles and genotype distribution of males and females were similar to the general population. Additionally, to determine the impact of +49A>G polymorphism on the progression of COVID-19 infection, we analyzed the genotypes of rs231775 polymorphism in COVID-19 patients with different disease severity. There was no significant difference between groups by conducting a multivariate logistic regression analysis. It should be noted that demographic parameters such as age, gender, and comorbidities are stratified according to genotypes of rs231775. There were no significant differences relating to rs231775.

Conclusion: More investigations are needed worldwide to prove the impact of this genetic variation on other ethnicities.

Keywords: COVID-19, CTLA-4, polymorphism, rs231775

Bioinformatic investigation of plant compounds as a potential inhibitor of the main protease of covid 19

Aref Yarahmadi[1], Behrooz Doosty[1]

1. Young Research and Elite Club, Iran.

Background: The SARS-COV-2 coronavirus has led to the spread of the respiratory disease covid-19, and due to the rapid spread of the disease, a drug is needed to treat covid-19. The current study aims to investigate various medicinal compounds using molecular docking and enzyme inhibition (6LU7) to take a step in the treatment of corona disease.

Methods: In this study, to examine how the compound binds to the active site of the enzyme, drawing the chemical structure of the compounds, energy optimization, Docking study, and final analyses from Discovery Studio, Chimera, Hyperchem, and online servers respectively Hdock was used. And the main protease enzyme of Covid-19 with the code (6L7U) was downloaded from the website (PDB) of the protein data bank and plant compounds were also received from the PUPCHEM website.

Results: The studied compounds can occupy the active site of the enzyme, and the binding energy level in kaempferol -142/93, in curcumin -147/13, in allicin -52/43, in ellagic acid -148/81, in cymarin -170/59, in thymol -83/77, in capsaicin -127/66, and coumarin -98/54. The most hydrogen bond between the (ligand and the receptor) was related to ellagic acid. Among the selected compounds, cymarin with the most negative binding energy was the best inhibitory compound.

Conclusion: Due to the relatively high effectiveness of the compounds in the bioinformatics study, for additional investigations, the effect of these compounds can be analyzed in vitro and in vivo.

Keywords: Coronavirus, COVID-19 main protease, Molecular docking, Herbal compounds

Broad spectrum reactivity of neutralizing mouse monoclonal antibodies against SARS-CoV-2 variants of concern

Faezeh Maghsood[1], Mohammad Mehdi Amiri[1], Mahmood Jeddi-Tehrani[2], Amir-Hassan Zarnani[3], Sylvie Schmidt[4], Géraldine Laumond[4], Christiane Moog[4], Fazel Shokri[5]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4. Université de Strasbourg, Laboratoire d'ImmunoRhumatologie Moléculaire, INSERM UMR_S 1109, Strasbourg, France
5. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin-converting enzyme-2 (ACE2) via receptor binding domain (RBD) of spike (S) protein for human cell entry. RBD is considered as a promising target for immunotherapy and vaccine design. Here, we characterized a panel of anti-RBD monoclonal antibodies (MAbs) isolated from RBD-immunized mice by hybridoma technology.

Methods: Epitope mapping using a panel of 20-mer overlapping peptides by enzyme-linked immunosorbent assay (ELISA) indicated the reactivity of several hybridomas towards restricted RBD peptide pools, with the dominance of peptides spanning amino acids 76-110 and 136-155.

Results: All neutralizing MAbs potently reacted with and neutralized the variants of concern (VOCs), including Alpha, Beta, Gamma, Delta, and Omicron by ELISA, pseudovirus-based neutralization test (PVNT), and conventional virus neutralization test (CVNT) at different levels. However, some MAbs were less sensitive to the emerging mutations within the VOCs, highlighting the necessity for the selection of suitable MAbs for passive immunotherapy.

Conclusion: Our data provide important information for understanding the immunogenicity of RBD, and the potential application of the novel neutralizing MAbs for passive immunotherapy of SARS-CoV-2 infection.

Keywords: COVID-19, Monoclonal antibodies, Neutralization, Omicron, SARS-CoV-2

Cellular and Humoral Immune Responses against AS03-Inactivated SARS-CoV-2 Vaccine

Mahla Abbasi[1], Fatemeh Roodbari[1], Akbar Khorasani[2], Mehdi Mahdavi[3]

1. Department of Microbiology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran.
2. Department of FMD Vaccine Production, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.
3. Advanced Therapy Medicinal Product (ATMP) Department, Breast Cancer Research Center, Motamed Cancer Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran.

Background: Several vaccines in the world are commercialized for COVID-19 infection. One of these vaccines is inactivated SARS-CoV-2 vaccine which is formulated in Alum adjuvant that is not capable of the induction of cellular immune responses. In the present study, inactivated SARS-CoV-2 virus was formulated in AS03 adjuvant and the immunogenicity was compared with the Alum-based vaccine.

Methods: Experimental 6-8-week-old BALB/c mice were immunized three times at three weeks intervals with AS03- and Alum-based vaccines along with PBS as a control group. Lymphocyte proliferation of spleen cells was performed by the BrdU method, IL-4 and IFN-γ cytokines were assessed on the spleen cell culture supernatant by quantitative ELISA kits. Specific total IgG and IgG1/IgG2a were assessed with an optimized indirect ELISA.

Results: The results showed that the vaccine formulated in AS03 adjuvant led to a significant lymphocyte proliferation response versus Alum-based and PBS control group.

Conclusion: In addition, the cytokines responses showed a Th1 platform in our vaccine formulation. Furthermore, IgG response showed an improvement versus the other experimental groups.

Keywords: inactivated vaccine, covid19, AS03, IFN-γ, IL-4

Characterizing the immune responses of subjects who survived or succumbed to COVID-19: Can immunological signatures predict outcome?

Hossein Motedayyen[1], Farshid Fathi[2], Nahid Eskandari[2]

1. Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran 6 Halal Research Center
2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Immunodeficiency has a pivotal role in the pathogenesis of coronavirus disease 2019 (COVID-19). Several studies have indicated defects in the immune system of COVID-19 patients at different disease stages. Therefore, this study investigated whether alters in the immune responses of COVID-19 patients may be considered as predicting factors for disease outcomes.

Methods: The percentages of innate and adaptive immune cells in the recovered and dead patients with COVID-19, and healthy subjects were determined by flow cytometry. The levels of pro- and anti-inflammatory cytokines and other immune factors were also measured by enzyme-linked immunosorbent assay.

Results: On the first day of hospitalization, the frequencies of CD56dim CD16+ NK cells and CD56bright CD16dim/-NK cells in patients who died during treatment were significantly increased compared to recovered and healthy individuals (p<0.0001). The recovered and dead patients had a significant increase in monocyte number in comparison with healthy subjects (p<0.05). No significant change was observed in Th1 cell numbers between the recovered and dead patients while Th2, Th17 cell, and Treg percentages in death cases were significantly lower than healthy control and those recovered, unlike exhausted CD4 + and CD8 + T cells and activated CD4+T cells (p<0.0001–0.05). The activated CD8 + T cell was significantly higher in the recovered patients than in healthy individuals (p<0.0001–0.05). IL-1α, IL-1β, IL-6, and TNF-α levels in patients were significantly increased (p<0.0001–0.01). However, there were no differences in TNF-α and IL-1β levels between dead and recovered patients. Unlike the TGF-β1 level, IL-10 was significantly increased in recovered patients (p<0.05). Lymphocyte numbers in recovered patients were significantly increased compared to dead patients, unlike the ESR value (p<0.001–0.01). CRP value in recovered patients significantly differed from dead patients (p<0.001).

Conclusion: Changes in frequencies of some immune cells and levels of some immune factors may be considered as predictors of mortality in COVID-19 patients.

Keywords: Immune cells, Pro-and anti-inflammatory cytokines, Immune dysregulation, COVID-19, Predicting factor

Chemokine CCL2 and chemokine receptor CCR2 in patients with COVID-19

Vahid Bagheri[1], Hossein Khorramdelazad[2], Mitra Abbasifard[3]

1. Immunology of Infectious Diseases Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
2. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Internal Medicine, Ali-Ibn Abi-Talib Hospital, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

Background: Due to the rapid spread and pandemic potential, coronavirus disease 2019 (COVID-19) has turned into one of the most serious public health problems in recent years. Despite the development of a number of antiviral drugs, most of them are not effective at the treatment of coronavirus disease 2019 (COVID-19) cytokine storm, and hyper-inflammation. Given that CCL2 is an important chemokine for regulating monocyte/macrophage trafficking in infection and inflammation, we designed this study to examine CCL2 serum levels and CCR2 expression in peripheral blood mononuclear cells (PBMCs) from COVID-19 patients of different age groups.

Methods: COVID-19 patients were diagnosed with clinical symptoms and confirmed by real-time polymerase chain reaction (RT-PCR) on nasopharyngeal swabs. All patients were divided into four age groups: group 1 (0-19 years), group 2 (20-40 years), group 3 (40-60 years), and group 4 (>60 years). CCL2 serum levels were measured using ELISA. CCR2 expression in PBMCs was evaluated by real-time PCR.

Results: In all age groups, CCL2 serum levels were significantly elevated in patients compared to healthy controls ($p< 0.0001$). CCL2 levels were higher in severe patients than in moderate patients. Moreover, CCR2 expression by PBMCs was higher in patients compared to control subjects. However, we observed a significant difference between patients and controls over 60 years of age ($p= 0.0353$). There was no significant difference in CCR2 expression between moderate and severe COVID-19 patients.

Conclusion: Our results emphasize the importance of the CCL2-CCR2 axis in SARS-CoV-2 infection. This chemokine axis could have a protective role in the early stage of COVID-19 by recruitment of monocytes/macrophages into the lung. However, excessive recruitment of immune cells into the lungs may cause hyper-inflammation and tissue damage at the late stage of the disease. Therefore, targeting the CCL2-CCR2 axis should be investigated in the different stages of SARS-CoV-2 infection.

Keywords: COVID-19, CCL2, CCR2, Chemokine

Chimerization and characterization of novel SARS-CoV-2 neutralizing mouse monoclonal antibodies

Ahmad Ghorbani[1], Mohammad Mehdi Amiri[1], Fazel Shokri[1], Hamidreza Yadegari[1], Faezeh Maghsood[1], Amir-Hassan Zarnani[1], Seyed Alireza Razavi[1], Mahmood Jeddi-Tehrani[2]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Monoclonal Antibody Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran

Background: Since the beginning of the COVID-19 pandemic in 2019, neutralizing antibodies have been widely used for disease prophylaxis and treatment. The major target for these neutralizing antibodies is the receptor-binding domain (RBD) of the viral spike protein. In our previous work, we developed a panel of neutralizing mouse monoclonal antibodies (mAbs) against the RBD protein. In the present study, we converted three of these neutralizing mAbs into chimeric mouse-human forms for therapeutic purposes.

Methods: Light and heavy chain variable regions of three murine monoclonal antibodies (m4E8, m3B6, m1D1) were amplified and ligated to human Cγ1 and Cκconstant region genes using splice overlap extension PCR (SOEing PCR). After cloning of heavy and light chains of each antibody sequence into a dual promoter mammalian expression vector, the final constructs were transiently expressed in DG-44 cells and the purified chimeric antibodies were characterized by ELISA, Western blotting, and flow cytometry. The neutralizing potency of the chimeric mAbs was determined by the surrogate virus neutralization test (sVNT), pseudovirus neutralization test (pVNT), and conventional virus neutralization test (cVNT) in vitro.

Results: The ELISA results showed that all three recombinant mAbs contained human Cγ1 and Cκ constant regions and were able to specifically bind to the RBD of SARS-CoV-2 with affinities comparable to the parental mAbs. Western blot analysis showed similar epitope specificity profiles for both the chimeric and the parental mouse mAbs. The results of sVNT, pVNT, and cVNT indicated that c4E8 had the most neutralizing activity with IC50 values around 1.772, 0.009, and 0.01 µg/ml, respectively. All chimeric and mouse mAbs displayed a similar pattern of reactivity with the SARS-CoV-2 variants of concern (VOC) tested, including Alpha, Delta, and the wild type.

Conclusion: The chimeric mAbs were successfully developed and displayed similar neutralizing potency as the parental mouse mAbs. These mAbs are potentially valuable tools for the control of COVID-19 disease.

Keywords: Chimeric antibody, SARS-CoV-2, COVID-19, immunotherapy, neutralizing antibody, RBD

Clinical Characteristics and Spike Glycoprotein Mutations in Iranian patients with Coronavirus Disease (COVID-19).

Shahrzad Ahangarzadeh[1], Laleh Shariati[2], Arezou Dabiri[3]

1. Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran
2. Department of Biomaterials, Nanotechnology and Tissue Engineering, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran
3. Applied Physiology Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran

Background: Mutations in spike glycoprotein which is a key protein of SARS-CoV-2, could have a direct impact on pathogenicity and virulence. The D614G mutation, a non-synonymous mutation at position 614 of the spike glycoprotein, is a dominant variant circulating worldwide. This experiment investigated the occurrence of mutations in the critical region of the spike gene and the relation of clinical symptoms with spike mutations in isolated viruses from Iranian COVID-19 patients during the second and third waves of the pandemic.

Methods: A total of 60 COVID-19 patients were considered to participate in a prospective study. RNA extraction, cDNA synthesis, and RT-PCR (in three overlapping fragments) were performed. Each patient's reverse transcriptase polymerase chain reaction (RT-PCR) products were assembled and sequenced. Information and clinical features of all sixty patients were collected, summarized, and analyzed using the GENMOD procedure of SAS 9.4.

Results: Nine nonsynonymous mutations were detected after analysis. The D614G was the most frequent mutation among the amino acid changes. In our study, D614G mutation was determined in 51.66% of patients. No significant relationship was observed for all the studied symptoms with the incidence of D614G mutation.

Conclusions: D614G had the highest frequency among the studied sequences, and its frequency increased significantly in the samples of the third wave compared to the ones of the second wave of the disease.

Keywords: COVID-19; clinical symptoms; spike glycoprotein; mutation; D614G

CNS demyelinating disease following inactivated or viral vector SARS-CoV2 2 vaccines

Narges Ebrahimi[1], Vahid Shaygannejad[2]

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Neurology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Several reports have been documented in possible association with the administration of different SARS-CoV-2 vaccines and CNS demyelinating disorders, specifically post-mRNA vaccines. We report twelve cases of developing Multiple sclerosis (MS) or Neuromyelitis Optica spectrum disorders (NMOSD) following neither the first nor second dose of inactivated or viral vector COVID-19 vaccine.

Methods: We retrospectively compiled twelve patients' medical information with a new onset of MS or NMOSD in their first six weeks following a COVID-19 vaccine.

Results: We report twelve cases of MS (n=9), CIS (n=1), and NMOSD (n=2) following COVID-19 inactivated vaccines (n=11) or viral vector vaccines (n=1) and, within some days following either the first (n=3), second dose (n=8), or third dose (n=1). Their median age was 33.3 years, ranging from 19 to 53. Ten were women (83%). All patients fully (n = 5) or partially (n = 2) recovered after receiving 3 doses of corticosteroids. Common medications were Natalizumab, Teriflunomide, Dimethyl fumarate, and rituximab. Also, Interferon beta was administered to one patient with severe symptoms of numbness.

Conclusion: Our case series identifies the Sinopharm BBIBP-CorV and the AstraZeneca AZD1222 vaccines as potential triggers for CNS demyelinating diseases. Vaccine administration routines are not affected by these rare and coincidental events. However, these manifestations are not deniable and require serious attention. Further investigations are needed to clarify the actual mechanisms and real associations.

Keywords: COVID-19 vaccine, vaccination, demyelinating disease, Multiple sclerosis, Neuromyelitis Optica spectrum disorders, NMOSD

Comparative evaluation of inflammatory indices in COVID-19 patients with different involvement of lung

Mohammad-Shafi Mojadadi[1], Ramin Shahraeeni[2], Abulfazl Shakiba[3], Ahmad Aram[4]

1. Department of Immunology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.
2. Department of Radiology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.
3. Department of Infectious diseases, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.
4. Student Research committee, Sabzevar University of Medical Sciences, Sabzevar, Iran.

Background: COVID-19 is an infectious disease that affects more than 200 countries and has different symptoms from mild to severe. Therefore, it is very important to investigate a fast, simple, and reliable method to help diagnose the COVID-19 infection to start treatment, until other available methods are validated. Therefore, the present study was conducted with the aim of a comparative investigation of inflammatory indices in COVID-19 patients with different lung involvement in Vasei Sabzevar Hospital.

Methods: The present study is cross-sectional and descriptive-analytical, in which 301 patients with COVID-19 (admitted to Vasei Hospital) were divided into different groups (Mild, Moderate, Severe) according to the severity of lung involvement based on the findings of the lung CT scan. This part of the work was done by a radiologist who is completely unaware of the CBC diff test results. Then, using the results of the complete blood cell count and differential test (CBC.diff) of the patients, inflammatory indices of neutrophil to lymphocyte, monocyte to lymphocyte, platelet to lymphocyte, and systemic inflammation index were calculated in patients. In the end, the average inflammatory indices of COVID-19 patients with different lung involvement were compared with each other using a one-way analysis of variance. The data was analyzed by SPSS software version 16 and the significance level was considered 0.05.

Results: In this study, 301 patients with COVID-19 who were hospitalized due to lung involvement were examined. Their average age was 59.32 ± 17.95 with an age range of 16 to 95 years. More than half of them (51%) were over 60 years old. According to CT scan findings, mild lung involvement was observed in 39%, moderate involvement in 37%, and severe lung involvement in 24% of patients. 13% of them died. Mortality among patients with mild, moderate and severe lung involvement was 2%, 5%, and 41%, respectively. There was no significant change in the number of platelets among patients in the three groups (p=0.24). The number of neutrophils was significantly higher in patients with severe lung involvement compared to the other two groups (p<0.001). The number of monocytes was significantly lower only in patients with moderate lung involvement compared to the mild involvement group (p<0.01). The number of lymphocytes in patients with severe lung involvement was significantly lower compared to the other two groups (p<0.01). The index of NLR, PLR, and systemic inflammation was significantly higher in patients with severe lung involvement compared to the other two groups. Also, the MLR index was significantly higher in patients with severe lung involvement compared to the moderate lung involvement group. 13% of patients infected with COVID-19 had died, 55% were men and 45% were women. No statistically significant difference was observed between mortality and gender of patients (p=0.73). There is a statistically significant difference between mortality and lung involvement (p< 0.001) and mortality in patients with severe lung involvement was significantly higher than other patients. There was no significant difference in the amount of hematological and inflammatory indices in patients with COVID-19 with different lung involvement between the two sexes (male and female) (p> 0.05).

Conclusion: The indices of NLR, PLR, MLR, and systemic inflammation are higher in patients with severe lung involvement compared to other groups with less involvement, and this shows the importance of these indices in determining the prognosis of COVID-19.

Keywords: COVID-19, inflammatory index, neutrophil, lymphocyte, platelet, monocyte

Comparative immunogenicity of four approved recombinant COVID-19 vaccines in BALB/c mice

Navid Dashti[1], Forough Golsaz-Shirazi[1], Mohammad Mehdi Amiri[1], Mehdi Mohammadi[1], Mahmood Jeddi-Tehrani[2], Fazel Shokri[3]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Background: Urgent demand for safe and effective vaccines to halt the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, led to the development of several vaccines within a short period of time. These vaccines were evaluated individually, but little is known about their comparative potency. Here, we report the comparative immunogenicity of four commonly used recombinant COVID-19 vaccines in Iran, including, Sinopharm, SpikoGen, Soberana Plus, and Noora in BALB/c mice.

Methods: Different groups of female BALB/c mice received 3 doses of each vaccine. The dose of each vaccine was selected based on the reported preclinical publications. The serum levels of antibodies against the vaccine formulation (anti-vaccine) as well as the viral receptor binding domain (anti-RBD) were measured by ELISA. The neutralization efficacy of each vaccine was evaluated by the surrogate virus neutralization test (sVNT), pseudotype virus neutralization test (pVNT), conventional virus neutralization test (cVNT), and flow cytometry.

Results: All vaccines induced seroconversion in immunized mice. High levels of anti-vaccine antibodies were induced by all vaccines, however, Soberana Plus and Sinopharm vaccines induced significantly higher levels of anti-RBD antibodies as compared to SpikoGen and Noora. These results were supported by the virus neutralization assays showing very weak and negligible neutralization potency by SpikoGen and Noora brands in all neutralization assays.

Conclusion: Our results indicate inefficient immunogenicity of the SpikoGen and Noora vaccines and suggest further comparative assessment of the potency and efficacy of these vaccines in vaccinated subjects.

Keywords: "COVID-19", "vaccine", "immunogenicity", "neutralization"

Comparing the Antibody Reactions to the AstraZeneca and Sinopharm Vaccines

Narges Karami[1], Enayat Anvari[2], Atefe Ghamar Talepoor[1], Iraj Ahmadi[2], Kurosh Kalantar[2]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Physiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran.

Background: Coronavirus disease 2019 (COVID-19) has been recognized as a global public health crisis by the World Health Organization. Effective vaccines are urgently needed to control Emerging Infectious Diseases. Knowledge about the development and duration of virus-specific antibodies after vaccination against infectious diseases such as COVID-19 is essential for figuring out how to use vaccines to control the pandemic. Our aim was to compare the antibody levels in healthy people who were not previously infected with SARS-CoV-2 and were vaccinated with AstraZeneca (ChAdOx1 nCoV-19) or Sinopharm (BBIBP-CorV) vaccine.

Methods: A comparison was made between 2 groups of participants who were divided according to the type of vaccine. 17 and 20 participants who were vaccinated with the AstraZeneca and Sinopharm respectively were included in this study. Four weeks after the first and second doses of vaccination-neutralizing antibody and anti-receptor-binding area (RBD) IgG levels were measured using enzyme-related immunosorbent assay (ELISA) techniques.

Results: Anti-(RBD) IgG levels were higher among AstraZeneca recipients than Sinopharm in both first (14.51 µg/ml vs. 1.160 µg/ml) and second (46.68 µg/ml vs. 11.43 µg/ml) doses. The same result was earned about the neutralizing Abs in which the titer of the antibody was higher in Astrazeneca recipients than Sinopharm subjects after the primary (7.77 µg/ml vs. 1.79 µg/ml, $p<0.0001$) and the second dose (10.36 µg/ml vs. 4.88 µg/ml, $p<0.0001$). Moreover, the concentrations of SARS-CoV-2 neutralizing antibody and anti-RBD IgG negatively correlated with the age of subjects who had received one dose and two doses of AstraZeneca ($p=0.031$; $r=-0.8149$; $p=0.01$; $r=-0.9011$; $p=0.04$; $r=-0.5196$ and $p=0.04$; $r=-0.5971$, respectively).

Conclusions: Our obtained results demonstrate that two doses of the AstraZeneca vaccine generated a higher antibody response compared to Sinopharm.

Keywords: COVID-19, Vaccine, Neutralizing antibody, Oxford-AstraZeneca, Sinopharm

Comparison of AstraZeneca and sinopharm vaccines as boosters in protection against COVID-19 infection

Saeed Motlaghzadeh[1], Arash Letafati[2]

1. Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: As the global number of confirmed cases rises past 640 million, vaccination remains the most effective measure in controlling COVID-19. Studies have shown that two doses of vaccination can significantly reduce hospitalization and mortality rates among patients, but the effectiveness of booster doses is also important. We aimed to evaluate the role played by the type of the 3rd dose of vaccination by comparing the safety and efficacy of two common vaccination histories differing only in the 3rd received dose.

Methods: We conducted a cross-sectional study on patients with respiratory symptoms suspected of having SARS-CoV-2 infection using Real-time PCR. We also collected information on the age, gender, and type of vaccine received for the third dose.

Results: Out of 346 cases with respiratory symptoms, 120 cases tested positive for SARS-CoV-2 and had received two doses of Sinopharm and a different booster dose of either AZD1222 (AstraZeneca) or BIBP (Sinopharm). Among these 120 patients, vaccination with AZD1222 as a booster dose resulted in fewer symptoms compared to those vaccinated with three doses of BIBP.

Conclusions: Our study demonstrates that booster doses can help reduce hospitalization and the severity of infection, and it appears that a combination of different vaccines may be effective against severe COVID-19 infection.

Keywords: COVID-19, SARS-CoV-2, Sinopharm, AstraZeneca

Comparison of the expression of miR223, NLRP3, and IL-1βgenes axis in patients with the severe form of COVID-19 and healthy individuals

Sheyda Houshmandfar[1], Ali Khodadadi[2], Mohammad-Reza Mahmoudian-Sani[3], Roohangiz Nashibi[4], Mohammad Rashno[5]

1. Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran Abadan University of Medical Sciences, Abadan, Iran
2. Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran 3. Cancer, Petroleum and Environmental Pollutants Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4. Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5.Department of Immunology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran 6. Cellular & Molecular Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: NLRP3 inflammasome hyperactivity during SARS-CoV2 infection is a key factor for severe inflammation in patients with COVID-19. Regarding NLRP3-inflammasome activation regulation, miR223 is among the key miRs, the present study was conducted to compare healthy individuals and patients with the severe form of COVID-19 in terms of the alterations of the expression of miR223, NLRP3, and IL-1βgenes axis.

Methods: This was a case-control study with the ethics code of IR.AJUMS.REC.1400.112 and grant number CMRC-0015. In total, 40 patients with the severe form of COVID-19 admitted to the infectious ward of Razi Hospital of Ahvaz, Iran, who were homogenous in terms of age (40 years) and gender, were selected based on study inclusion and exclusion criteria. Real-time PCR was used to assess the expression of miR223, NLRP3, and IL-1βgenes using blood samples of the patients. Finally, data analysis was carried out in SPSS and PRISM software.

Results: The mean ± standard deviation of the age of the participants in the case and control groups were reported to be 49.4±10.59 and 49.52±7.61 years, respectively. According to the results, the expression of the IL-1βgene was 3.9 times higher in patients with COVID-19, compared to the control group (p=0.0005). Moreover, the expression of the NLRP3 gene was 6.04 times higher in the case group, compared to the control group (p<0.0001). On the other hand, the expression of miR-223 was 5.37 times lower in the case group, compared to the control group (p=0.04).

Conclusion: Our findings were indicative of the potential role of miR-223 and the dysregulation of NLRP3 inflammasome followed by IL-1βas a regulatory factor in the pathogenesis of COVID-19 as other inflammatory diseases. According to the results of the study, miR-223 could be used as a diagnostic biomarker and a potential therapeutic target in patients with COVID-19.

Keywords: COVID-19, Inflammasome, miR-223, cytokine storm

COReNAPCIN: The first Iranian mRNA vaccine.

Vahid Khoddami[1]

1. ReNAP Therapeutics, Tehran, Iran.

Background: At the forefront of the biopharmaceutical industry, messenger RNA (mRNA) technology offers a flexible and scalable platform to address the urgent need for worldwide immunization in pandemic situations. This strategic powerful platform has recently been used to immunize millions of people proving both safety and the highest level of clinical efficacy against infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Methods: Here we provide the preclinical report of COReNAPCIN®; a vaccine candidate against SARS-CoV-2 infection. COReNAPCIN® is a nucleoside-modified mRNA-based vaccine formulated in lipid nanoparticles (LNPs) for encoding the full-length prefusion stabilized SARS-CoV-2 spike glycoprotein on the cell surface.

Results: Vaccination of C57BL/6 and BALB/c mice and rhesus macaque with COReNAPCIN® induced strong humoral responses with high titers of virus-binding and neutralizing antibodies. Upon vaccination, a robust SARS-CoV-2 specific cellular immunity was also observed in both mice and non-human primate models. Additionally, vaccination protected rhesus macaques from symptomatic SARS-CoV-2 infection and pathological damage to the lung upon challenging the animals with high viral loads of up to 2×108 live viral particles.

Conclusion: Overall, our data provide supporting evidence for COReNAPCIN® as a potent vaccine candidate against SARS-CoV-2 infection for clinical studies. To further evaluate the safety of the candidate vaccine, COReNAPCIN® is now testing in clinical trial phase I.

Keywords: SARS-COV-2, mRNA vaccine, vaccine

Correlation between HSP27 gene expression levels and severity of symptoms in patients with COVID-19

Fereshteh Ebrahimi[1], Mohsen Salehi[2], Shahram Arsang-Jang[3], Hossein Dinmohammadi[4], Akbar Mohammadzadeh[5]

1. Department of Medical Genetics and Molecular Medicine, School of Medicine, Zanjan university of Medical Sciences, Zanjan, Iran
2. Department of Emergency Medicine, School of Medicine, Zanjan university of Medical Sciences, Zanjan, Iran
3. Department Of Biostatistics & Epidemiology, School of Medicine, Zanjan university of Medical Sciences, Zanjan, Iran
4. Department of Medical Genetics and Molecular Medicine, School of Medicine, Zanjan university of Medical Sciences, Zanjan, Iran
5. Department of Medical Genetics and Molecular Medicine, School of Medicine, Zanjan university of Medical Sciences, Zanjan, Iran

Background: SARS-CoV-2, a newly emerged RNA virus, is the causative agent of the COVID-19 pandemic. There is still no complete information about the molecular pathogenesis of SARS-CoV-2 infection. In the meantime, one of the attractive issues is to investigate heat shock proteins (HSPs) as one of the host factors in this disease. They play a crucial role in many signaling pathways such as inflammation and also in various diseases, especially viral infections. HSP27 plays anti-apoptotic, antioxidant, and cell cycle-promoting roles. Although HSP27 is involved in a wide range of viral infections, its role during SARS-Cov-2 infection is unclear. The current study provides insight into the changes in HSP27 gene expression in patients with mild and moderate-to-severe symptoms of COVID-19 compared to the healthy control group.

Methods: In this study, 102 samples of patients with COVID-19 (54 patients with moderate to severe symptoms and 48 patients with mild symptoms) and 42 samples as the healthy control group were selected. After total RNA extraction from the samples and cDNA synthesis, the relative expression level of HSP27 and GAPDH (as an internal control) was performed with a Real-Time PCR reaction. Finally, the data were analyzed using the Pfaffl method. Statistical analyzes were performed using R software version 4.2.

Results: Based on the results obtained indicate the average expression of the HSP27 gene in patients with mild symptoms (1.11 ± 0.66) and patients with moderate to severe symptoms (1.17 ± 0.73), no statistically significant difference was observed compared to the control group (1.09 ± 0.42) (95% confidence interval value).

Conclusions: Although the changes in HSP27 gene expression were not statistically significant in patients with COVID-19 compared to the control group, it seems that conducting further studies could help clarify the importance of HSP27 in SARS-CoV-2 infection.

Keywords: COVID-19, HSP27, SARS-CoV-2, Heat Shock Proteins

Cross-talk between heat shock proteins and SARS-CoV-2; A systematic review

Fereshteh Ebrahimi[1]

1. Department of Medical Genetics and Molecular Medicine, School of Medicine, Zanjan university of Medical Sciences, Zanjan, Iran.

Background: Heat Shock Proteins are important factors in regulating cell survival, differentiation, and cell death. Some of them participate in innate and adaptive immunity. They are crucial factors in many signal transduction pathways such as inflammation. So, the control of HSPs expression would be a powerful tool for the invading virus to manipulate the fate of the host cell. As well, studies show that there are interactions between HSPs and viruses and they can be involved in the viral life cycle. This review will mainly focus on the host stress response process to SARS-CoV-2 infection and the therapeutic potential of targeting these processes.

Methods: This systematic review was conducted to outline comprehensive studies published in PubMed, Scopus, Science Direct, and Google Scholar databases from 2019 to 2022 by using keywords 32 articles were screened, and 11 were totally included.

Results: We comprehend that HSPs may be associated with the outcome of infection with SARS-Cov2 and can attract great interest as potential antiviral targets. HSP60 enhances inflammatory response with pro-inflammatory cytokine induction so inhibition of that could ameliorate inappropriate inflammatory reactions in severe COVID-19 patients. Hsp70 limited NF-κB activation, thereby suppressing inflammation. Thus, Hsp70 expression may indirectly regulate COVID-19 pathology. Hsp90 is involved in the replication of various human coronaviruses. To this end, inhibitors of Hsp90 are of interest as possible therapies against COVID-19. Another HSP called GRP78 may stabilize the interaction between the viral spike protein and the cellular host receptor to facilitate entry or serve as an alternative host factor for viral entry. Also, elevated plasma HSP72 concentrations and blunted HSR in critically ill patients with severe COVID-19 pneumonia were described.

Conclusion: Understanding the interaction between HSPs and COVID-19 infection may contribute to the prognosis and treatment of this disease. Although the standing of some of them is not yet clearly defined.

Keywords: HSP, COVID-19, SARS-CoV-2

Cross-Talk of Brain Immunology and Long Covid-19 Syndrome: Systematic Review

Pegah Moharrami Yeganeh[1], Kimia Esmaeilzadeh[1]

1. Zanjan University of Medical Sciences, Zanjan, Iran.

Background: Long Covid-19 Syndrome refers to a set of symptoms that generally present after recovery from an acute Covid-19 infection. The presentations include various respiratory, gastrointestinal, musculoskeletal, and neurological symptoms, with neurological symptoms being the most common. Understanding how brain immunology affects and is affected by this syndrome sheds new light on the management of Long Covid-19. This study aims to examine the correlation between Brain Immunology and Long Covid-19 Syndrome.

Methods: In this systematic review, we searched Google Scholar, PubMed databases, and Scopus and found 63 articles with specific keywords. (Long Covid-19 Syndrome, Brain Immunology, Neurological Symptoms, Neuroimmunity). A total of 21 articles were selected based on our exclusion criteria.

Results: The reviewed documents demonstrated that there are a number of neurological processes that contribute to the development of Long Covid-19 Syndrome. Mood changes, dizziness, cognitive difficulties, memory loss, headache, fatigue, attention deficit, and confusion have been reported as common neurological symptoms associated with long covid. It has been speculated that neurological manifestations of Long Covid might be due to the direct passing of the virus through the blood-brain barrier and affecting cervical structures, such as medullary structures. Neurological symptoms have been linked to the activation of specific toll-like receptors (TLRs) by the Covid-19 virus that disturbs the cytokine network. Dysregulating the cytokine network and the upregulation of some pro-inflammatory cytokines such as Tumor necrosis factor (TNF)-alpha, Interleukin (IL)-6, and IL-1-beta has also been proposed as possible contributing factors of neurological symptoms in long covid syndrome.

Conclusion: The exact neuropathology of Long Covid-19 Syndrome still needs more studies and harder evidence. We believe that these results can provide a backbone for neuromodulator drugs to be considered in the treatment of Long Covid-19 Syndrome.

Keywords: Long Covid-19 Syndrome, Brain Immunology, Neurological Symptoms, Neuroimmunity

Design of a multi-epitope-based peptide vaccine against the S and N proteins of SARS-COV-2 using immunoinformatics approach

Seyedeh Zeinab Hosseini[1], Arian Karimi Rouzbahani[2], Farnaz Kheirandish[3]

1. Department of Bacteriology and Virology Shiraz University of Medical Sciences.
2. Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran.
3. Department of Medical Parasitology and Mycology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

Background: As the new pandemic created by the COVID-19 virus created the need for rapid acquisition of a suitable vaccine against SARS-CoV-2 to develop Immunity and reduce mortality, this study aimed to identify SARS-CoV-2 S protein and N antigenic epitopes by using immunoinformatic methods.

Methods: to design a vaccine against SARS-CoV-2, for which S and N protein-dependent epitopes are predicted B cell, CTL, and HTL were determined based on antigenicity, allergenicity, and toxicity that were non-allergenic, non-toxic, and antigenic and were selected for the design of a multi-epitope vaccine structure. Then, to increase the safety of Hbd-3 and Hbd-2 as adjuvants, they were connected to the N and C terminals of the vaccine construct, respectively, with a linker. The three-dimensional structure of the structure was predicted and optimized, and its quality was evaluated. The vaccine construct was ligated to MHCI. Finally, after optimizing the codon to increase expression in E. coli K12, the vaccine construct was cloned into a pET28a (+) vector.

Results: Epitopes that were used in our survey were based on non-allergenic, non-toxic, and antigenic. Therefore, 543-amino-acid-long multi-epitope vaccine formation was invented by linking 9 cytotoxic CTL, 5 HTL, and 14 B cell epitopes with appropriate adjuvants and connectors that can control the SARS coronavirus 2 infection and could be more assessed in medical scientific researches.

Conclusion: We believe that the proposed multi-epitope vaccine can effectively evoke an immune response toward SARS-CoV-2.

Keywords: SARS-CoV-2, Multi-epitope, Vaccine, Immunoinformatic, Antigenicity

Designing a multi-epitope vaccine against SARS-COV-2 targeting the RBD domain and monitoring with biosensors: an immunoinformatics approach

Omid Yeganeh[1], Yasamin Pahlavan[2], Chiman Karami[3]

1. Department of Microbiology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran
2. Biosensor Sciences and Technologies Research Center, Ardabil University of Medical Sciences, Ardabil, Iran.
3. Department of Microbiology, Parasitology and Immunology, Ardabil University of Medical Sciences, Ardebil, Iran.

Background: One of the ways to find a suitable vaccine against SARS-CoV-2 is to examine structural proteins, especially spike proteins. In the present study, a multi-epitope vaccine was designed using immunoinformatics approaches that stimulate both CD4 and CD8 T cell immune responses against the SARS-CoV-2 spike proteins. Epitope vaccine candidates were designed using B and T cell epitopes and can act as immunogens and stimulate immune responses in the host immune system.

Methods: RNA was extracted from 40 patients' throat swab samples, and a PCR assay was carried out to detect the receptor-binding domain (RBD) using forward and reverse primers. The Big Dye Terminator v3.1 Cycle Sequencing Kit was used to sequence positive results from the PCR amplification in both directions. Then, the sequence of the RBD region was sent to GenBank to obtain the accession number. Physical properties of secondary structure, homology modeling, epitopes 3D structure, and antigenicity prediction were carried out using IEDB, PSIPRED, and Vaxijen online tools, respectively.

Results: Physicochemical properties of amino acid sequence RBD region were predicted using the online tool ProtParam of ExPASy bioinformatics resource portal. The secondary structure of the amino acid sequences was predicted by PSIPRED v3.3 online tools. Homology modeling and 3D structure prediction were obtained using MolProbity version 4.4. This sequence showed that the MolProbity score of this sequence was 2.17 and its clash score was 7.04. Ramachandran Favored was 84.85% and Ramachandran Outliers was 1.43% with C-Beta Deviations 3, Bad Bonds 5.602 and Bad Angel 5.806 and Twisted Prolines 1.2, QM -6.71.

Conclusion: Evaluation of designed B and T cell epitopes showed a high immunogenicity score, indicating that they are promising candidates for multi-epitope vaccine development. The epitopes presented in this study may help to develop an effective vaccine against SARS-CoV-2.

Keywords: Immunoinformatics, COVID-19, Epitope, Vaccine, Peptide, SARS-CoV-2, T cell, B cell

Designing an efficient multi-epitope peptide vaccine candidate against SARS-CoV-2 by in silico method

Zahra Yazdani[1], Alireza Rafiei[2], Mohammadreza Yazdani[3], Reza Valadan[4]

1. Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. 2. Students Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
3. Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, Iran
4. Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: We implemented an immunoinformatic approach to design an efficient multi-epitope vaccine against SARS-CoV-2.

Methods: Immunodominant epitopes from structural proteins of a spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins of SARS-CoV-2 were selected based on the stimulation of humoral and cellular immunity, antigenicity ability, and allergenicity probability. The selected epitopes aligned between SARS-CoV-2 and SARS species. We used appropriate adjuvants in the vaccine structure to potentiate the immunogenicity of the antigens. The vaccine segments were connected by appropriate linkers. The physicochemical properties, structural stability, and immunological characterizations of the vaccine were evaluated. Modeling, refinement, and validation were performed to access a high-quality three-dimensional structure of the vaccine protein. Docking evaluation showed an appropriate interaction between the vaccine and toll-like receptors (TLRs) 3, 5, 8, and angiotensin-converting enzyme 2 (ACE-2). In silico cloning showed that the vaccine could be effectively expressed in E. coli.

Results: The designed vaccine construct consists of several immunodominant epitopes from structural proteins of SARS-COV-2. These peptides promote cellular and humoral immunity and interferon-gamma responses. Also, these epitopes have a high antigenic capacity and are not likely to cause allergies. To enhance the vaccine immunogenicity, we used three potent adjuvants: Flagellin of Salmonella enterica subsp. enterica serovar Dublin, a driven peptide from high mobility group box 1 as HP-91, and human beta-defensin 3 protein. The vaccine has suitable physicochemical properties, high-quality structure, and the ability to stimulate the immune system and interact with TLRs 3, 5, 8, and ACE-2. Also, in silico cloning demonstrated that the vaccine can be efficiently expressed in E. coli.

Conclusion: Totally, a potential vaccine candidate with proper immunological and stable physicochemical properties against SARS-CoV-2 was designed. It is expected the vaccine could be capable to protect humans from COVID-19 disease.

Keywords: SARS-CoV-2, multi-epitope peptide vaccine, humoral immunity, cellular immunity

Determinant factors of COVID-19 vaccine acceptance by the parents of children aged 5 to 12 years, North Khorasan, Iran

Maryam Nezafat Firizi[1], Hasan Namdar Ahmadabad[1], Shahin Mafinezhad[2]

1. Department of Pathobiology and Laboratory Sciences, North Khorasan University of Medical Sciences, Bojnurd, Iran
2. Department of Pediatrics, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

Background: Although the COVID-19 vaccination program for children aged 5-12 is ongoing in Iran, the level of acceptance by parents has yet to be significant. In the present study, the factors related to the acceptance of the Covid-19 vaccine by the parents of children aged 5 to 12 years were investigated.

Methods: This web-based cross-sectional study was conducted on 340 parents of children aged 5-12 in Bojnord City. The data collection tool of the study included the demographic profile form and the attitude and awareness form about the COVID-19 vaccine for children. Descriptive statistics, chi-square, and independent t-tests were used to analyze the data. The significance level of the p-value was considered less than 0.05.

Results: More than 60% of parents did not want to vaccinate their children against covid-19. Most parents declared the vaccine's dangerousness as their most important concern (45.9%). Most parents evaluated their knowledge and awareness about the covid-19 disease and its vaccine at an average level (57.4%). There was no significant relationship between the level of parents' knowledge and awareness of the acceptance of the COVID-19 vaccine (p<0.05). There was a significant relationship between the indicators of gender, age, education, and the level of trust in the health and treatment system with parents' willingness to vaccinate their children (p<0.05).

Conclusion: The fear of not being safe for children with the COVID-19 vaccine is the main reason for parents' negative acceptance of the vaccine. It is suggested to consider programs to improve the knowledge and awareness of parents about the COVID-19 vaccine and its safety among parents.

Keywords: Acceptance, COVID-19, vaccine, children, parents,

Different Formulations of Inactivated SARS-CoV-2 Vaccine Candidates in Human Compatible Adjuvants: Potency Studies in Mice Showed Different Platforms of Immune Responses

Melika Haghighi[1]

1. Recombinant Vaccine Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Background: Several inactivated SARS-CoV-2 vaccines have been approved for human use, but are not highly potent. In this study, different formulations of the inactivated SARS-CoV-2 virus were developed in Alum, Montanide 51VG, and Montanide ISA720VG adjuvants, followed by an assessment of immune responses.

Methods: The SARS-CoV-2 virus was inactivated with formalin and formulated in the adjuvants. BALB/c mice were immunized subcutaneously with 4 µg of vaccines on days 0 and 14; (IL-4) and (IFN-g), cytotoxic T lymphocyte (CTL) activity, and specific immunoglobulin G (IgG) titer and IgG1, IgG2a, and IgG2a/IgG1 ratio, and anti-receptor-binding domain (RBD) IgG response were assessed 2 weeks after the final immunization.

Results: Immunization with SARS-CoV-2-Montanide ISA51VG showed a significant increase in the IFN-γcytokine versus SARS-CoV-2-Alum, SARS-CoV-2-Montanide ISA720VG, and control groups (p<0.0033). Cytokine IL-4 response in the SARS-CoV-2-Alum group showed a significant increase compared with SARS-CoV-2-Montanide ISA51VG, SARS-CoV-2-Montanide ISA720VG, and control groups (p<0.0206). In addition, the SARS-CoV-2-Montanide ISA51VG vaccine induced the highest IFN-γ/IL-4 cytokine ratio versus other groups (p<0.0004). CTL activity in SARS-CoV-2-Montanide ISA51VG and SARS-CoV-2-Montanide ISA720VG groups showed a significant increase compared with SARS-CoV-2-Alum and control groups (p<0.0075). Specific IgG titer in SARS-CoV-2-Montanide ISA51 VG and SARS-CoV-2-Montanide ISA720VG showed a significant increase compared with SARS-CoV-2-Alum and control groups (p<0.0143). Results from specific IgG1and IgG2a in SARS-CoV-2-Alum, SARS-CoV-2-Montanide ISA51VG, and SARS-CoV-2-Montanide ISA720VG vaccine showed a significant increase compared with phosphate buffer saline (PBS) group (p< 0.0001), but SARS-CoV-2-Montanide ISA51VG and SARS-CoV-2-Montanide ISA 720VG groups showed the highest IgG2a/IgG1 ratio and a significant increase compared with SARS-CoV-2-Alum group (p<0.0379). Moreover, inactivated SARS-CoV-2+Alum and SARS-CoV-2-Montanide ISA 720VG groups demonstrated a significant increase in anti-RBD IgG response versus the SARS-CoV-2-Montanide ISA51VG group.

Conclusuin: It seems that the type of vaccine formulation is a critical parameter, influencing the immunologic pattern and vaccine potency and human-compatible oil-based adjuvants were more potent than Alum adjuvants in the vaccine formulation.

Keywords: SARS-COVID, Alum, Vaccine, Adjuvant

Effect of quercetin supplement on clinical factors and miR-218 expression in hospitalized patients with severe covid-19

Marziye Shamshiri[1], Roohangiz Nashibi[2], Ali Asadirad[3], Sara Iranparast[3], Ali Khodadadi[3]

1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3.Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: MIR-218 has a negative regulatory role in the antiviral immune response by reducing the expression of RIG-I. Also, the usage of quercetin as an anti-inflammatory drug in this pathway can Help us to improve the use of complementary treatments for the recovery of Covid patients. In this study, the therapeutic effectiveness of quercetin in combination with specific antiviral treatments was investigated to evaluate the ability of quercetin to prevent the progression of the disease to the critical stage and reduce the levels of inflammatory factors related to the pathogenesis of SARS-Cov-2, in hospitalized patients with severe covid-19.

Methods: Through an open-label clinical trial, 20 severe cases were randomly selected and considered as a control group for comparison with the intervention group. Patients in the experimental group were treated with quercetin at 1000 mg/body daily for 7 days in addition to antiviral drugs. According to the results, the consumption of quercetin was significantly associated with the change of mir-218 and the reduction of serum levels of ESR, q-CRP, and LDH in the intervention group.

Results: In addition, although the values were within the normal range, the statistical output showed a significant increase (p value=0.000) in the level of respiratory rate in patients taking quercetin. Based on our observations, quercetin is safe and effective in altering mir-218 and reducing serum levels of ESR, q-CRP, and LDH as important markers involved in the severity of COVID-19.

Conclusion: However, according to the observed results and the investigation of the mortality rate of covid patients in different stages, as well as further investigation of the performance of this drug, further studies can be useful to compensate for the limitations of our study and clarify the therapeutic potential of quercetin in the treatment of 19-Covid.

Keywords: COVID-19, Quercetin

Efficacy of Guluronic Acid (G2013) in the Management of Hospitalized COVID-19 Patients; a Randomized, Positive Control Clinical Trial.

Zahra Aghazadeh[1], Davod Sanaee Delir[2], Hamid Reza Gholamrezaie[2], Arezoo Sadoughi[1], Amir Nezami Asl[3], Mehran Noori Sanami[2], Armita Mahdavi Gorabi[3], Younes Panahi[4], Mahsa Taeb[1], Alireza Razavi[1], Sara Rafia[1], Zahra Naderiyan[2], Behrouz Robat-Jazi[1], Abbas Mirshafiey[1]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
2. Farhikhtegan Hospital, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
3. Health Research Center, Chamran Hospital, Tehran, Iran
4. Deputy for Research and Technology, Ministry of Health and Medical Education, Tehran, Iran

Background: Since 2019, SARS-CoV-2 (COVID-19) infection, with a vast spectrum of clinical and paraclinical symptoms, has been a major health concern worldwide. Therapeutical management of COVID-19 includes antiviral and anti-inflammatory drugs. NSAIDs, as the second-line therapy, are often prescribed to mitigate or prevent disease symptoms and complications of COVID-19 which are mostly caused by immune system responses. The a-L-guluronic acid (G2013) is a non-steroidal patented (PCT/EP2017/067920) agent with immunomodulatory properties. This study investigated the effect of G2013 on the outcome of COVID-19 in moderate to severe hospitalized patients.

Methods: The disease's symptoms were followed up during hospitalization and for 4 weeks' post-discharge in G2013 and control groups. Paraclinical indices were tested at the time of admission and discharge. Statistical analysis was performed on clinical and paraclinical parameters and ICU admission and death rate.

Result: The primary and secondary outcomes indicated the efficiency of G2013 on COVID-19 patients' management. There were significant differences in the duration of improvement of fever, coughing, fatigue/malaise. Also, a comparison of paraclinical indices at the time of admission and discharge showed significant change in prothrombin, D-dimer, and platelet. As the main findings of this study, G2013 significantly decreased the percentage of ICU admission (control: 17 patients, G2013:1 patient) and death (control: 7 cases, G2013:0).

Conclusion: These results allow the conclusion that G2013 has sufficient potential to be considered for moderate to severe COVID-19 patients, can significantly reduce the clinical and physical complications of this disease, has a positive effect on modulating the coagulopathy process, and aids in saving lives.

Keywords: Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, COVID-19, α-L-guluronic acid, G2013

Evaluation of anti-SARS-CoV-2 RBD IgG response after third booster dose of SpikoGen® in individuals with two previous doses of Sinopharm

Amirhossein Hajijafari[1], Somayeh Malekmohammad[2], Peyman Bemani[1]

1. Department of Immunology, School of Medicine, University of Isfahan, Isfahan, Iran.
2. Department of Microbiology, School of Medicine, University of Hamadan Hamadan, Iran.

Background: Coronavirus disease 2019 (COVID-19) vaccines have been rapidly developed globally as a measure to combat the disease. Although these vaccines have been demonstrated to confer significant protection, there have been reports of temporal decay in antibody levels. Thus, COVID-19 vaccine boosters or third doses are recommended for adolescents and adults who completed their initial vaccination course more than 6 months prior. The aim of this study was to evaluate the level of IgG antibody against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein after the third dose of the SpikoGen® vaccine in individuals with two previous doses of the Sinopharm vaccine.

Methods: A total of 95 healthy individuals who had received two doses of the Sinopharm vaccine participated in this study. Anti-RBD IgG level was measured in the serum of participants before and 3 weeks after receiving the SpikoGen® vaccine as the third dose, using the ELISA method.

Results: 63 (66.3%) of the participants were female with a median age of 35 (range: 18-55) years and 32 (33.7%) were male with a median age of 36 (range: 18-56) years. A significant increase in the anti-RBD IgG level was observed in the vaccinees after the third booster dose of the SpikoGen® (p<0.0001). The mean anti-RBD IgG level before the third dose vaccination was 116.18 (SD ± 116.31) RU/mL (relative unit per milliliter) and after 3 weeks was 275.21 (SD ± 365.25) RU/mL. There was no difference between males and females in the case of anti-RBD IgG levels in response to the third dose of vaccination (p= 0.850).

Conclusion: SpikoGen® vaccine induced a robust IgG antibody response as a third booster dose in healthy individuals.

Keywords: SARS-CoV-2, Anti-RBD IgG, SpikoGen®, Vaccine

Evaluation of clinical and laboratory findings in asthmatic patients with COVID-19 admitted to the Ardabil Imam Khomeini hospital

Aylin Tarighi[1], Ahmad Ghorbani[2], Shahnaz Hossienzadeh[3], Mohammadreza Aslani[1], Hassan Ghobadi[1]

1. Department of pathology, School of Medicine, Ardabil University of Medical Science
2. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Microbiology, Parasitology and Immunology, Ardabil University of Medical Sciences, Ardabil, Iran

Background: Considering the coronavirus pandemic, the role of underlying diseases in the severity of COVID-19 prognosis seems to be significant. One of the underlying diseases is asthma, which is thought to affect the severity of COVID-19 due to the similar nature of these two diseases. In this study, we have evaluated the clinical and laboratory findings of Asthmatic patients suffering from COVID-19 and compared the results of recovered patients with deceased in Ardabil Imam Khomeini Hospital.

Methods: It was a retrospective cross-sectional study conducted from the beginning of April to the end of September 2020 on asthmatic patients with covid-19 admitted to Imam Khomeini Hospital in Ardabil. Inclusion criteria were all patients with asthma who tested positive for COVID-19 using real-time PCR or who were diagnosed based on CT scan findings according to national guidelines. A checklist containing demographic characteristics, and clinical and laboratory findings were completed for all cases. Questionnaire information was collected and analyzed using SPSS V21 software, and the results were analyzed using Fisher's exact test with $P<0.05$.

Results: Eighty-two asthmatic patients with COVID-19 were included in the study with a mean age of 54.67 ± 17.13 years, of which 37 were male (45.7%) and 44 were female (54.4%). At the end of the study, 72 patients (88.9%) recovered and 9 patients (11.1%) died due to the disease. The most common comorbidities in asthmatic patients with COVID-19 were hypertension (24.7%), diabetes (12.3%), cardiovascular disease (7.4%), and myocardial infarction (4.9%), respectively. Analysis of laboratory results revealed that neutrophil counts ($p<0.001$), PTT ($p<0.05$), INR ($p<0.05$), AST ($p<0.05$), LDH ($p<0.001$), Ferritin ($p<0.001$), BS ($p<0.001$), and Urea ($p<0.001$) in asthmatic deceased individuals were higher than recovered patients.

Conclusion: Our results indicate that monitoring inflammatory, metabolic, and liver biomarkers in serum can reasonably facilitate the early prognosis of asthmatic patients with COVID-19 for further healthcare interventions.

Keywords: Asthma, COVID-19, Clinical findings, laboratory results, comorbidity

Evaluation of possible association between serum levels of aldosterone and cortisol with clinical symptoms progression in COVID-19 suspicious outpatients tested for SARS-CoV2 RTPCR: an analytical cross-sectional study

Sahar Golabi[1], Mahshid Naghashpour[2], Hamid Ghiassian[3], Sara Mobarak[4], Maryam Adelipour[5], Maghsud Piri[6], Maryam Seyedtabib[7]

1. Department of Medical Physiology, School of Medicine, Abadan University of Medical Sciences, Abadan, Iran
2. Department of Nutrition, School of Medicine, Abadan University of Medical Sciences, Abadan, Iran
3. School of Medicine, Abadan University of Medical Sciences, Abadan, Iran
4. Department of Infectious Diseases, School of Medicine, Abadan University of Medical Sciences, Abadan, Iran
5. Department of Biochemistry, School of Medical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
6. Vice Chancellor for Health, Abadan University of Medical Sciences, Abadan, Iran

*7Department of Biostatistics and Epidemiology, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran***Background**: Aldosterone is a key component of the Renin-Angiotensin-Aldosterone System (RAAS). The RAAS could play a substantial role in the pathophysiology of COVID-19. Also, the dynamics of the Hypothalamic-Pituitary-Adrenal (HPA) axis may have changed in COVID-19. As an essential factor in assessing immune system activity, cortisol is an important part of this axis. The present study compared the serum levels of aldosterone and cortisol in COVID-19 outpatients with those of potentially non-infected participants. We also aimed to assess the possible association between serum levels of aldosterone and cortisol with clinical symptom progression in COVID-19 outpatients.

Methods: This epidemiological health service center-based cross-sectional-analytical study was designed and conducted at Abadan University of Medical Sciences, Abadan, Khuzestan Province (southwestern Iran) between the beginning of April and the end of July 2020. Demographic (sex, age) and clinical (SPO2, respiratory rate, heart rate) data were collected. Serum cortisol and aldosterone measurements were done using the ELISA technique. Clinical symptoms of the positive PCR group were followed up on for 28 days in weekly intervals.

Results: SPO2 was significantly lower in the positive PCR group, but the respiratory rate was significantly higher (p=0.03 and p=0.001, respectively). We found significantly higher levels of aldosterone in males of the negative PCR group in comparison with females (p=0.05). Cortisol (OR=0.937, p=0.033) and aldosterone (OR=1.005, p=0.020) levels had a decreasing and increasing effect on the chances of respiratory symptoms occurring over time, respectively. Also, over time, women were twice as likely as men to develop neurologic symptoms (OR=0.530, p=0.015).

Conclusion: Our findings revealed that cortisol and aldosterone are associated with the chance of respiratory symptoms occurring over time. However, the levels of these two markers do not seem to be related to the progression of clinical symptoms of lower grades of COVID-19.

Keywords: COVID-19, Aldosterone, Cortisol, Clinical symptoms progression

Evaluation of rheumatoid arthritis antibodies in patients with Covid-19 in Salmas County

Dorna Rafighi[1], Javid Taghinejad[2], Zahra Nikbakht[3]

1. Ph.D Student, Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Microbiology, Malekan Branch, Islamic Azad University, Malekan, Iran.
3. Department of Microbiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

Background: Rheumatoid arthritis is a chronic systemic inflammatory disease with unknown causes that if not diagnosed and treated in time can lead to severe complications and disability. Inflammatory diseases are on the rise due to the impact of the global pandemic of Covid-19 on the immune system. The purpose of this study is to evaluate the frequency of rheumatoid arthritis in patients with Covid-19 in Salmas County.

Research method: This study was conducted from the winter of 2020 to the fall of 2021. A total of 50 patients with Covid-19 with previous and unprecedented clinical signs of rheumatism were referred to a medical diagnostic laboratory for blood sampling after visiting a physician. Serums of clients were tested with an Anti-CCP kit by ELISA method and Individuals were evaluated in two groups, SPSS Statistics V22.0 was used for data analysis employing T-test and Kolmogorov-Smirnov tests.

Results: Out of 30 patients with positive antibody titers in the age range of 30 to 65 years, 17 (56.66%) were female and 13 (43.33%) were male with a mean age for positive ones of 47.60 ± 10.087 and a variance of 101.76. 11 patients (36.66%) had a previous history of rheumatoid arthritis and 19 patients (63.33%) had no history of the disease. The age groups of 40-35 years and 60-55 years were high-risk groups.

Conclusion: According to the present study and considering previous studies, economic factors, nutrition, inappropriate occupations, and quality of life can be named as the causes of rheumatoid arthritis. Since Covid-19 disease mainly affects the immune system and causes widespread inflammation in the body, factors such as nutrition and quality of life contribute to the onset of the disease in Covid-19 patients.

Keywords: Rheumatoid Arthritis, Anti-CCP, Covid-19, Salmas

Evaluation of Serum Total IgA in Severe and Mild COVID-19 Patients Compared to Control Group

Maral Barzegar-Amini[1], Farahzad Jabbari-Azad[1], Mahmoud Mahmoudi[2], Maliheh Dadgarmoghaddam[3], Faramarz Farzad[4], Ali Qaraee Najafabadi[5]

1. Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Community Medicine Department, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Department of Immunology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
5. Young Researchers and Elites Club, Islamic Azad University, Mashhad Branch, Mashhad, Iran.

Background: Considering the critical role of IgA in respiratory infectious disease and the lack of any scientific research on the association between IgA and COVID-19 patients, in the present study, we aimed to evaluate total serum IgA levels in severe and mild COVID-19 patients.

Methods: In this cross-sectional study, 216 definite severe COVID-19 patients (inpatient group), 183 positive specific COVID-19 IgG with mild or no symptoms (outpatient group), and 203 healthy subjects with negative specific serology (control group) were investigated. Laboratory and clinical features were collected and analyzed by SPSS software version 22. Statistical tests including Independent Samples T-test, ANOVA test, and Post Hoc test were performed.

Results: The mean±SD of IgA in all subjects was 2.23±0.78 (g/L). Statically significant changes in IgA between the three groups were observed (p<0.05). This difference was significant between the outpatient and inpatient groups (p<0.05). The mean±SD of serum IgG in all subjects was 15.83±5.73 (g/L). Strong statically significant changes were seen in IgG between all three groups (p<0.001). There was a significant negative correlation between IgG and IgA total titers of the outpatient group (p=0.011*r=-0.188).

Conclusion: In conclusion, total serum IgA and IgG level is significantly associated with the severity of COVID-19 infection. Also, total serum IgA and IgG correlation was associated with the severity of illness. Of note, a Low level of IgA is asymptomatic and highly frequent in Iran and other countries. We suggest the evaluation of serum IgA levels in high-risk people and strengthening the immune system in low levels of IgA subjects, to reduce the rate of death. Oral or nasal in combination with parenteral vaccination, are recommended due to increasing immunity versus COVID-19 by the further secretion of the IgA antibody and preventing virus transmission.

Keywords: Serum IgA Level, Severe COVID-19, Mild COVID-19

Evaluation of the interaction Between Tumor Growth Factor-βand Interferon Type I Pathways in Patients with COVID-19: Focusing on ages 1 to 90 years

Hosssein Khorramdelazad[1]

1. Iran University of Medical Sciences, Tehran, Iran.

Background: Evidence revealed that age could affect immune responses in patients with the acute respiratory syndrome of coronavirus 2 (SARS-CoV-2) infection. In this study, we investigated the role of age on immune responses, especially on the interaction between the tumor growth factor-β (TGF-β) and interferon Type-I (IFN-I) axes in the pathogenesis of novel coronavirus disease 2019 (COVID-19).

Methods: This is an age-matched case-control investigation enrolling 41 COVID-19 patients and 40 healthy controls categorized into four groups, including group 1 (up to 20 years), group 2 (20-40 years), group 3 (40-60 years), and group 4 (more than 60 years). Blood samples were collected at the time of admission. The expression of TGF-βRI, TGF-βRII, IFNARI, IFNARII, interferon regulatory factor 9 (IRF9), and SMAD3 was measured using the real-time PCR technique. In addition, serum levels of TGF-β, IFN-α, and SERPINE1 were assessed by the enzyme-linked immunosorbent assay (ELISA).

Results: All biomarkers were measured and analyzed in 4 age groups. The expression of TGF-βRI, TGF-βRII, IFNARI, IFNARII, IRF9, and SMAD3 was markedly upregulated in all age groups of patients compared with the matched control groups. Additionally, serum levels of IFN-αand SERPINE1 were significantly higher in patient groups than in control groups. While TGF-βserum levels were only significantly elevated in the 20 to 40 and more than 60 years' patient group than in matched control groups.

Conclusion: These data demonstrated that the age of patients, at least at the time of admission, does not significantly affect TGF-βand IFN-I-associated immune responses. However, the severity of the disease can affect SERPINE1 levels.

Keywords: COVID-19, TGF-β, Interferon, Fibrosis

Evaluation of the relationship between three IL-10 genotypes (rs1800896 (-1082 T>C), rs1800871 (-819 A>G), rs1800872 (-592 T>G)) with the severity of COVID-19

Hossein Azadinejad[1], Parisa Feizollahi[2], Alireza Rezaiemanesh[2], Farhad Salari[2], Mohammad Hossein Zamanian[3], Ali Gorgin Karaji[4]

1. Student Research Committee, School of Medicine, Kermanshah University of Medical, Sciences, Kermanshah, Iran.
2. Department of Immunology, School of medicine, Kermanshah University of medical sciences, Kermanshah, Iran..
3. Department of Infectious diseae, School of medicine, Kermanshah University of medical sciences, Kermanshah, Iran.
4. Department of Immunology, School of medicine, Kermanshah University of medical sciences, Kermanshah, Iran.

Background: The severity of coronavirus disease 2019 (COVID-19) is associated with increased levels of inflammatory cytokines as well as IL-10. The aim of this study was to investigate the relationship between three IL-10 genotypes (rs1800896 (-1082 T>C), rs1800871 (-819 A>G), rs1800872 (-592 T>G)) with the severity of COVID-19.

Material and methods: 143 patients with severe and 150 patients with mild forms of COVID-19 from the Kermanshah province of Iran participated in this study. Briefly, blood was taken from the patients and DNA was extracted from the blood. Then the polymerase chain reaction-restriction fragment length peptide (PCR-RFLP) technique was used to determine the genotypes of IL-10.

Results: Our results showed that the frequency of all three genotypes (rs1800896 (-1082 T>C) rs1800871 (−819 A>G), and rs1800872 (−592 T > G)) in patients with the severe form of COVID-19 was not significantly different compared to those with mild form of COVID-19 (P-value = 0.91, 0.08 and 0.1 respectively). In addition, the evaluation of the alleles of these three genotypes showed that the frequency of the alleles of the genotypes was not significantly different between the two groups (P-value = 0.78, 0.16, and 0.07, respectively).

Conclusion: Overall, the results of our study showed that there is no relationship between the severity of COVID-19 and the frequency of IL-10 genotypes (rs1800896 (-1082 T>C), rs1800871 (-819 A>G) and rs1800872 (-592 T>)) and alles.

Keywords: COVID-19, IL-10 genotypes, Severity of COVID-19

Evaluation of two type I IFN-induced genes (ly6e and usp18) gene expression and association with the severity of clinical symptoms in COVID-19 patients

Umoulbanin Alibakhshi[1], Sara Ghaffarpour[2], Mohammad Mehdi Naghizadeh[3], Tooba Ghazanfari[3]

1. Department of immunology, Faculty of Medicine, Shahed University, Tehran, Iran
2. Immunoregulation Research Center, Shahed University, Tehran, Iran
3. Non-communicable Diseases Research Center, Fasa University of Medical Science, Fasa, Iran

Background: Covid-19 disease is a pandemic with high spreading power and a wide range of clinical symptoms and the lack of definitive treatment is a challenge for mankind in recent years. The antiviral response of type 1 interferon against the SARS-COV-2 virus is one of the most important pathways of the innate immune system in dealing with this disease. The purpose of this study is to investigate the relationship between the expression of two genes, ly6e, and usp18, with the severity of clinical symptoms in patients with covid-19.

Methods: Peripheral blood samples were prepared from patients and their DNA was extracted, then the expression of ly6e and usp18 genes was measured by real-time PCR method. cDNA samples were first amplified using specific primers. Data analysis was performed using chi-square, Kruskal-Wallis, and Mann-Whitney tests. A significance level of 0.05 was considered.

Results: The expression of the ly6e gene was significantly ($p=0.008$) decreased in the hospitalized group compared to the control group. No significant difference was seen in the expression of the usp18 gene among the studied groups.

Conclusion: Proper expression of ly6e protein can be related to controlling the severity of clinical symptoms of covid-19 disease.

Keywords: Keywords: covid-19, severity of clinical symptoms, type one interferon, ly6e, usp18

Formulation of Inactivated SARS-CoV-2 Virus in MF-59 adjuvant; Analysis of Cellular and Humoral Immune Responses

Tadeh Sepanian[1], Rasoul Baharlou[1], Akbar Khorasani[2], Mehdi Mahdavi[3]

1. Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran.
2. Department of FMD Vaccine Production, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.
3. Advanced Therapy Medicinal Product (ATMP) Department, Breast Cancer Research Center, Motamed Cancer Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran.

Background: Vaccination is the most rationale way in the prevention of infectious disease. Various vaccines are developed to prevent infectious diseases. But, it is proven that adjuvant and vaccine formulation is a critical parameter in the immunogenicity and vaccine efficacy. In this study, inactivated SARS-CoV-2 virus was formulated in MF-59 adjuvant and the potency of this vaccine in the induction of cellular and humoral immune responses was assessed in experimental mice.

Methods: Experimental BALB/c mice were immunized subcutaneously, three times with MF-59- and Alum-based vaccines. In addition, a PBS as a control group was considered. Three weeks after the vaccine immunization, lymphocyte proliferation of spleen cells was performed by the BrdU method, and IL-4 and IFN-γ cytokines were assessed on the spleen cell culture supernatant by quantitative ELISA kits. Furthermore, specific total IgG and IgG1/IgG2a were assessed with an optimized indirect ELISA.

Results: The results of the present study show that immunization with the MF-59-inactivated SARS-CoV-2 vaccine lead to robust cellular and humoral immune responses which showed significant differences versus the Alum-based vaccine and PBS control group.

Conclusion: It seems that MF-59 could be used as a suitable adjuvant in SARS-CoV-2 vaccine development.

Keywords: SARS-CoV-2, MF-59, Vaccine, BALB/c, Immune response

Hemoperfusion reduces the serum level of IL-6 in COVID-19 patients

Mohammad Bakhshivand[1], Farid Gorbaninezhad[1], Somaye Mirzayi[2], Haleh Mikaeili[3]

1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. ghorbaninezhadfarid@gmail.com
3. Tuberculosis and Lung Disease Research Center, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Unregulated production of inflammatory factors, such as interleukin (IL)-6, has been implicated as the primary pathogenic mechanism of coronavirus disease-19 (COVID-19). In this study, we evaluated the effect of hemoperfusion (HP) on serum levels of IL-6 in COVID-19 patients.

Methods: Between April 2020 and June 2020, 35 severe COVID-19 patients were enrolled in this investigation at the Imam-Reza Hospital, Tabriz University of medical sciences. We used the HA330 disposable HP cartridge (Jafron Biomedical Company, China). After a hemodialysis catheter was placed, patients underwent HP for at least three days in a row. Each HP session lasted for four hours. The blood flow rate and a heparin dosage were 180 to 250 ml /minute and 10-12 units per kg per hour, respectively throughout the HP process.

Results: A significant diminishment in serum IL-6 values in COVID-19 patients was found following HP ($p\leq$ 0.001). Also, the efficacy of this strategy was demonstrated by a reduction in IL-6 levels after HP (area under the curve (AUC) =0.7102 and p=0.0025).

Conclusion: According to our findings, HP may be a promising therapeutic option for COVID-19.

Keywords: COVID-19, IL-6, Hemoperfusion

HLA-A*11, -B*50, and -C*07, IL-6, and CRP are associated with Long Covid-19 Syndrome: A Cross-sectional study

Ensiyeh Torki[1], Arezou Gharezadeh[1], Marjan Moradi[3], Hamed Fouladseresht[1]

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Genetics, School of Science, Shahrekord University, Shahrekord, Iran

Background: Long-COVID-19 syndrome (LCS) is the persistence of symptoms and complications for more than 3-months of COVID-19 post-onset. Ongoing aberrant immune responses are believed to be responsible for these persistent complications. Due to diverse symptoms, there is no unified clinical definition for the diagnosis and prognosis of LCS. Hence, we investigated the association of HLA-I alleles, serum levels of Interleukin-6 (IL-6) and C-reactive-protein (CRP), and other laboratory parameters in LCS cases to determine diagnostic and prognostic molecular targets.

Methods: Demographic, clinical, and paraclinical data of 88 LCS cases (LCS+ group) and 96 COVID-19-recovered individuals without LCS (LCS- group) were collected during sampling. Laboratory parameters, including serum IL-6 and CRP levels, were measured. Low-resolution-Olerup-SSP-HLA-typing-kits also was used to determine HLA-I alleles.

Results: The individuals with a positive history of severe COVID-19 (SC) were more frequent in LCS+ ("LCS+ SC", n=49) than in LCS- ("LCS- SC", n=11; $p<0.001$). Serum CRP and IL-6 levels were significantly higher in LCS+ than in LCS- (p=0.005 and p=0.042; respectively). In the comparison among 4 groups, higher serum levels of CRP and IL-6 were evaluated in "LCS+ SC" compared to "LCS+ NSC" (p=0.0004 for CRP and $p<0.001$ for IL-6) and "LCS- NSC" ($p<0.001$ for both). Meanwhile, higher frequencies of HLA-A*11, -B*14, -B*38, -B*50, and -C*07 alleles and lower frequencies of HLA-A*32, -B*51, -C*08, and -C*12 alleles were observed in LCS+ compared to LCS- (for all $p<0.05$). Also, HLA-A*11, -B*14, -B*38, and -C*07 alleles were significantly more frequent in "LCS+ SC" compared to "LCS- NSC" (for all $p<0.05$). Whereas HLA-A*32, -B*51, and -C*08 alleles were lower (for all $p<0.05$). The higher serum CRP and IL-6 levels were associated with HLA-A*11, -B*50, and -C*07 alleles (for CRP $p>0.05$ and IL-6 $p<0.05$).

Conclusion: A positive history of severe COVID-19, serum IL-6, CRP levels, and HLA-A*11, -B*50, and -C*07 alleles jointly increase LCS risk.

Keywords: HLA Class I Genes, Long COVID-19 Syndrome, Interleukin-6, C-reactive protein

HLA-DRB1*01, TNF-α, and anti-β2GPI-IgG are associated with chronic COVID-19 syndrome: A Cross-sectional study

Arezou Gharezadeh[1], Ensiyeh Torki[1], Marjan Moradi[2], Hamed Fouladseresht[1]

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Department of Genetics, School of Science, Shahrekord University, Shahrekord, Iran

Background: HLAs are polymorphic molecules that have the determining role in the resolution or chronicity of infection complications. This may be important for chronic CVID-19 syndrome (CCS), the most challenging issue post-COVID-19. CCS is a clinical condition with various symptoms for months after SARS-CoV-2 infection, making it difficult to diagnose and monitor. Hence, to introduce molecular and inflammatory markers in CCS, the allele frequency of HLA-II was investigated in relation to inflammatory biomarkers.

Methods: We recruited 88 newly diagnosed CCS patients ("CCS") and 96 age-matched COVID-19-recovered individuals ("NCCS"). Serum TNF-α and anti-β2GPI-IgG levels were measured by ELISA technique and reported as Median [IQR1-IQR3]. A Low-resolution SSP-PCR method was used to determine HLA-DRB1, -DQB1, and -DPB1 alleles frequencies.

Results: Of 88 "CCS", 49 cases had a positive history of severe-critical COVID-19 (SC) ("CCS SC"), and of 96 "NCCS", 11 individuals were SC ("NCCS SC") (OR=9.70[95%CI=4.55-20.67], p<0.001). Compared to "NCCS", a higher frequency of HLA-DRB1*01 (OR=2.89[95%CI=1.095-7.619], p=0.026) and a lower frequency of HLA-DRB1*11 (OR=0.553[95%CI=0.341-0.898], p=0.016) were observed in "CCS". Considering the history of COVID-19 severity in each group, the HLA-DRB1*01 was more frequent in "CCS SC" compared to the "NCCS NSC" (OR=4.18[95%CI=1.53-11.38], p=0.005). In contrast, HLA-DRB1*11 was lower (OR=0.39[95%CI=0.21-0.74], p=0.004). The serum TNF-α and anti-β2GPI-IgG levels were higher in "CCS" than in "NCCS" (3.97[2.58-15.17] vs. 2.85[1.03-4.78], p=0.028 for TNF-α and 1.68[1.37-1.98] vs. 1.46[1.03-1.87], p=0.004 for anti-β2GPI-IgG). Also, the serum anti-β2GPI-IgG level was significantly higher in "CCS SC" (1.84[1.44-2.24]) compared to "CCS NSC" (1.61[1.23-1.76], p=0.036) and "NCCS NSC" (1.46[0.98-1.86], p=0.014). Whereas serum TNF-αlevel was no significantly higher in "CCS SC" (4.50[3.14-52.27]) than in other groups ($p>0.05$). The higher serum TNF-α and anti-β2GPI-IgG levels were measured in HLA-DRB1*01+ individuals (26.42[0.74-70.03] and 1.80[1.32-1.97]; respectively) compared to HLA-DRB1*01- (3.52[1.49-8.08], p=0.256 for TNF-α and 1.61 [1.21-1.91], p=0.336 for anti-β2GPI-IgG; respectively).

Conclusion: A positive history of severe COVID-19, HLA-DRB1*01, serum anti-β2GPI-IgG, and TNF-αlevels are positively associated with CCS.

Keywords: HLA Class II alleles, Chronic COVID-19 syndrome, Beta 2-Glycoprotein I, TNF-α

How asthmatic children dealt with the coronavirus pandemia? Adherence to Controller Medications and Level of Control among Children with Asthma Registered in Mashhad University of Medical Sciences

Ahmad Habibi Dashtebayaz[1], Nasrin Moazzen[2], Hamid Ahanchian[1], Zahra Abbasi Shaye[3], Alireza Peyvandi[4]

1. Clinical Research Development Unit of Akbar Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Community Medicine, Treatment Affairs, Mashhad University of Medical Sciences, Mashhad, Iran
4. Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Background: The coronavirus disease 2019 pandemic had a great effect on the lives of asthmatic children. In this study, we assessed changes in medication adherence and asthma control rate among our registered children with pediatric asthma.

Methods: This cross-sectional study was conducted on 113 patients registered in our asthma and allergy clinic in Mashhad, Iran. We called them via phone and completed a questionnaire on the level of asthma medication adherence and the asthma control test (ACT) before and in the 6 months after COVID-19 emergence. We investigated the changes in medication adherence and asthma control due to the COVID-19 pandemic.

Results: A total of 113 asthmatic children in the age range of 1 to 15 (mean, 7.02±3.24 years) were included in the study. There were zero confirmed positive cases among them since the COVID-19 introduction. The minority of patients (8.8%) had weak adherence, most of the children (35.4%) completed one course of prescribed medications, 33% had longer adherence, and only 25 patients (22.1%) had full adherence during the pandemic. Overall, our patients experienced better asthma control with a 1.51 rise in ACT score to arrive at 23.64 points out of 25. They had no asthma exacerbation or emergency visits. Over half of the families compiled the national preventive measures; as 85% of children had followed the stay-at-home order during the first peak of the pandemic.

Conclusion: Our patients managed to come to a higher asthma control level despite their generally decreased adherence to medications during the pandemic. COVID-19 not only could not worsen asthma status in our children but surprisingly improved it. This shows that preventive measures should be strongly applied to the asthmatic population.

Keywords: pediatric asthma, COVID-19, asthma control, medication adherence

IgY based therapeutic approaches in COVID-19: A Systematic Review

Mohammad Javad Yazdanpanah[1]

1. Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran.

Background: The SARS-CoV-2 or COVID-19 pandemic has been a very challenging health issue for medical society globally. Various preventive and curative methods have been suggested and tested for managing this destructive disease. Among recent research literature, Immunoglobulin Y (IgY) is evaluated as a new immunological targeted therapy. IgY is a homologue antibody to human IgG, obtained from chicken egg yolk. Several studies have investigated different therapeutic applications of IgY. This review study aimed to look over the efficiency of IgY antibodies in the treatment of COVID-19 by different approaches.

Methods: Data collection of the current study was based on a systematic search in valid databases like PubMed, Scopus, and Google Scholar. All articles containing keywords "IgY", "Immunoglobulin/Antibody Y", "egg yolk immunoglobulin/antibody", "SARS-CoV-2", "COVID-19" and "Corona Virus" in the title, published during the past ten years (2012-2022), have been included; then abstracts and main texts were scanned.

Results: Applications of Immunoglobulin Y regarding the treatment of COVID-19 have been explored extensively and its potential advantages and disadvantages have been studied. The efficiency of the IgY as a suitable therapeutic tool has been demonstrated in multiple articles. IgY is very safe and easy to provide. Neutralizing activity of this antibody has been proven as it is effective in virus entry and replication inhibition through interaction with the S protein and ACE2 receptor of the body cells. IgY can be used as either a monoclonal or polyclonal antibody against the SARS-CoV-2 virus in different forms of administration. This antibody can also be used in diagnosis and detection of the virus and even prophylactic use of IgY is an option through passive immunization.

Conclusion: IgY is a promising therapeutic tool that can be efficiently useful in the prevention, management, and treatment of the COVID-19 pandemic and probably other highly transmissible infectious diseases of viral or any origin causing serious health issues.

Keywords: COVID-19, SARS-CoV-2, Egg Yolk Antibody, Immunoglobulin Y

IL-10 and TGF-β1 serum levels are associated with disease severity in COVID-19 patients

Fatemeh Keshavarz[1], Ghasem Ghalamfarsa[2]

1. Department of Immunology, Shahid Beheshti University of medical scieneces, Tehran, Iran.
2. Cellular and Molecular Research Center, Yasuj University of Medcal Sciences, Yasuj, Iran.

Background: Accumulating pieces of clinical and experimental evidence revealed that COVID-19 patients often show increased levels of cytokine which is termed a cytokine storm. Accordingly, the aim of this study was to evaluate the serum levels of interleuki10 (IL-10), and TGF-β1 in patients infected with COVID-19.

Methods: The study consisted of 79 patients with laboratory-confirmed SARS-CoV-2 infection including 47 men and 32 women, ranging in age from 24 to 96 years. Moreover, COVID-19 patients were categorized into moderate and severe groups according to the clinical guidelines. IL-10 and TGF-β1 serum levels were determined by a human enzyme-linked immunosorbent assay (ELISA) kit.

Results: Serum IL-10 concentration was significantly higher in COVID-19-severs patients than in moderate cases ($P < 0.001$). Moreover, moderate patients had significantly higher levels of TGF-β1 compared to severe cases. The results showed that IL-10 was positively correlated with the CRP level and negatively correlated with total lymphocytes count.

Conclusion: Our data suggest that IL-10 and TGF-β1 serum levels are deregulated in patients with COVID-19. Further investigations with larger sample sizes and in different populations are needed to confirm our results.

Keywords: COVID-19; Cytokines; IL-10, TGF-β1; Disease severity

Immune changes during COVID-19 recovery play important role in clarifying disease severity

Hossein Motedayyen[1], Farshid Fathi[2], Nahid Eskandari[2]

1. Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran
2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Coronavirus disease 2019 (COVID-19) is largely associated with dysregulation and impairment of the immune system. This study investigated how immune system changes were related to disease severity in COVID-19 patients.

Methods: The frequencies of different immune cells and levels of pro- and anti-inflammatory cytokines in the whole blood of participants were determined by flow cytometry and enzyme-linked immunosorbent assay, respectively.

Results: The values of other inflammatory agents were also studied. In the late recovery stage, unlike CD56 high CD16+/− NK cells and monocytes, CD56low CD16+ NK cell numbers were increased (p< 0.0001–0.05). Th1, Th2, and Th17 cell percentages were significantly lower in patients than in healthy control (p<0.0001–0.05), while their frequencies were increased following disease recovery (p <0.0001–0.05). The numbers of Tregs, activated CD4+ T cells, and exhausted CD8+ T cells were significantly decreased during a recovery (p<0.0001–0.05). No significant change was observed in exhausted CD4+ T cell number during a recovery (p> 0.05). B cell showed an increased percentage in patients compared to healthy subjects (p<0.0001-0.05), whereas its number was reduced following recovery (p<0.0001–0.05). IL-1α, IL-1β, IL-6, TNF-α, and IL-10 levels were significantly decreased in the late recovery stage (p<0.0001–0.05). However, the TGF-β1 level was not significantly changed during the recovery (p>0.05). Lymphocyte numbers in patients were significantly decreased (p<0.001), unlike the ESR value (p<0.001). Lymphocyte number was negatively correlated to ESR value and Th2 number (p<0.05), while its association with monocyte was significantly positive on the first day of recovery (p<0.05).

Conclusion: The immune system changes during the disease recovery to improve and regulate immune responses and thereby may associate with the reduction in disease severity.

Keywords: COVID-19, disease recovery, disease severity, immune system, immunodysregulation

Immunogenicity and safety of the COVID-19 vaccines in adult patients with autoimmune inflammatory rheumatic diseases: A systematic review and meta-analysis

Safa Tahmasebi[1], Mina Alimohammadi[1]

1. Shahid Beheshti University of medical sciences, Tehran, Iran.

Background: COVID-19 vaccines approved by the FDA have been studied mainly in healthy individuals and there is limited information on their immunogenicity and safety in individuals with autoimmune diseases. Therefore, in this meta-analysis study, we comprehensively investigated the immunogenicity and safety of these vaccines in patients with autoimmune inflammatory rheumatoid diseases (AIIRD).

Methods: A literature comprehensive search was performed on various databases, including PubMed, Google Scholar, Web of Science, Scopus, and Embase to select cohort and clinical-trial (RCT) studies up to January 2022. Also, for quality assessment and heterogeneity tests of the selected studies, the PRISMA checklist protocol and the I2 statistic were used, respectively. Fixed and random-effects models were estimated based on the heterogeneity tests, and pooled data were determined as the standardized mean difference (SMD) with a 95% confidence interval (CI).

Results: As a result, we found that vaccines can cause favorable immunogenicity in vaccinated AIRD patients with an acceptable safety profile; however, older age and the concomitant consumption of glucocorticoids, rituximab, mycophenolate mofetil (MMF), and methotrexate (MTX) drugs could significantly reduce the vaccine immunogenicity.

Conclusion: Consequently, our findings revealed a significant humoral response (seropositive) and no apparent side effects in AIRD patients following the administration of COVID-19 vaccines.

Keywords: COVID-19, Vaccine, AIIRD, Safety, efficacy

Immunomodulatory effects of Nanocurcumin on NK cell responses in COVID-19 patients

Safa Tahmasebi[1], Majid Ahmadi[2]

1. Shahid Beheshti University of medical sciences, Tehran, Iran.
2. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Background: The COVID-19 pandemic has so far claimed over Four million lives globally. Moreover, the ongoing pandemic threatens to become a global humanitarian crisis since no aspect of human life has remained untouched by it. NK cells are capable of launching direct attacks on virus-infected cells as well as modulating immune response via cytokine production. Therefore, it is necessary to examine their role in SARS-CoV-2 infection. Hence, the immunomodulatory effect of Nanocurcumin was investigated in the present study in an attempt to counterbalance the immune response and improve the patient's clinical symptoms.

Methods: 60 confirmed COVID-19 patients and 60 healthy controls enrolled in the study. COVID-19 patients were divided into Nanocurcumin and placebo groups. NK cell frequency, cytotoxicity, and receptor gene expression were evaluated in both groups before and after the intervention.

Results: The results indicated that the Nanocurcumin with immunomodulatory function could increase the frequency of NK cells and their cytotoxic function in COVID-19 patients than in the placebo group by upregulating the expression of activating receptors and downregulating inhibitory receptors on these cells.

Conclusion: As an immunomodulatory agent, Nanocurcumin may be a helpful choice in order to NK cell function improve in COVID-19 patients and it may improve the clinical outcome of patients.

Keywords: COVID-19, SARS-CoV2, Natural killer cell, Cytotoxicity, Nanocurcumin

Improvement of the inactivated SARS-CoV-2 vaccine potency through formulation in alum/naloxone adjuvant; Robust T cell and anti-RBD IgG responses

Melika Haghighi[1]

1. Recombinant Vaccine Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Backgrounds: SARS-CoV-2, emerging as a major threat to public health, has to be controlled through vaccination. Naloxone (NLX), an opioid receptor antagonist, demonstrated its adjuvant activity for microbial vaccines. In this study, inactivated SARS-CoV-2 was developed in the Alum/NLX adjuvant to increase the potency of the inactivated SARS-CoV-2 vaccine.

Methods: BALB/c mice were immunized on days 0 and 14 with inactivated SARS-CoV-2-Alum, -Alum + NLX 3 mg/kg, -Alum + NLX 10 mg/kg, and -Freund adjuvant, as well as PBS. IFN-γand IL-4 cytokines and Granzyme-B release were assessed with ELISA. In addition, specific total IgG, IgG1/IgG2a isotypes, and ratio as well as anti-RBD IgG responses were assessed with an optimized ELISA.

Results: SARS-CoV-2-Alum-NLX10 group showed a significant increase in the IFN-γ cytokine response versus SARS-CoV-2-Alum, SARS-CoV-2-Alum-NLX3, and PBS groups. The SARS-CoV-2-Alum-NLX3 group exhibited a significant decrease in IL-4 cytokine versus SARS-CoV-2-Alum. The mice immunized with SARS-CoV-2-Alum-NLX10 showed a significant increase in CTL activity versus SARS-CoV-2-Alum and PBS. In addition, mice immunized with SARS-CoV-2-Alum-NLX3, SARS-CoV-2-Alum-NLX10, and SARS-CoV-2-Freund demonstrated an increase in IgG response, as compared with SARS-CoV-2-Alum and PBS group. Furthermore, all formulations of SARS-CoV-2 vaccines could induce both IgG1 and IgG2a isotypes. But, the IgG2a/IgG1 ratio in SARS-CoV-2-Freund and SARS-CoV-2-Alum-NLX10 revealed an increase as compared with that of the SARS-CoV-2-Alum group. Anti-RBD IgG response in the SARS-CoV-2-Alum-NLX10 group showed a significant increase as compared with the Alum-based vaccine.

Conclusion: Formulation of inactivated SARS-CoV-2 virus in NLX/alum adjuvant improved the potency of humoral and, especially, cellular responses.

Keywords: Alum Adjuvant, Immune Responses, Inactivated SARS-CoV-2 – virus, Naloxone Vaccine formulation

In-depth analysis of the type I Interferon signaling pathway and immunological response in hospitalized COVID-19 patients

Mohammad Sadegh Soltani-Zangbar[1], Javad Ahmadian Heris[2], Farzaneh Hasani[3], Mehdi Yousefi[4]

1. 1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. 2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
2. 3. Department of Allergy and Clinical Immunology, Pediatric Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.
3. 1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
4. 1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. 2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Background: In the third decade of the twenty-first century, the COVID-19 pandemic has emerged as the primary threat to human existence in the world. To develop effective treatments and vaccines, numerous investigations on the pathophysiology and structure of the SARS-CoV-2 virus have been conducted. In this work, we sought to compare individuals with mild and severe COVID-19 disease in terms of their immunological phenotype and IFN-I signaling pathways.

Methods: For this study, 100 COVID-19 patients were included, 50 of whom had mild symptoms and 50 of whom had severe ones. Using flow cytometry, the frequency of B, Treg, Th17, CD8+ T, and CD4+T lymphocytes in addition to NK cells was assessed. Using real-time PCR and western blotting, it was possible to examine the expression of interferon regulatory factors (IRF) 3&7 as well as downstream signaling molecules for IFN-I, such as STAT-1, STAT-2, TYK-2, and JAK-1 at both the protein and RNA levels. By using an enzyme-linked immunosorbent test, immune cytokine levels, including those for IL-1, TNF-α, IL-6, IL-2R, IL-17, IL-10, and IFN-I, as well as anti-IFN-autoantibodies presence, were assessed.

Results: Patients with COVID-19 had significantly lower absolute counts of NK cells, CD4+T, CD8+ T, and B lymphocytes as a consequence of immune phenotyping. Th17 cell frequency and Treg cell frequency both showed a striking rise and fall, respectively. In COVID-19 patients with severe symptoms compared to healthy persons, all signaling molecules of the IFN-I downstream pathway and IRFs, which are constituted of STAT-1&2, IRF-3&7, TYK-2, and JAK-1 showed substantially low expression levels at both the protein and RNA levels. Anti-IFN-autoantibodies were found in the sera of 14 out of 50 patients with severe problems, compared to 0 and 2 for healthy individuals and patients with mild symptoms, respectively.

Conclusion: According to our findings, immune cell dysregulation and the presence of anti-IFN-autoantibodies are positively correlated with the degree of illness in COVID-19 patients. In-depth research is required to learn more about this situation.

Keywords: IFN-I, Illness severity, signaling pathway, COVID-19, Immune-phenotype

Interferons expression by T Lymphocytes of COVID-19 patients, upon stimulation with toll-like receptor (TLR) agonists and SARS-CoV-2 antigens

Samaneh Abdolmohammadi-Vahid[1], Behzad Baradaran[2], Armin Sadeghi[3], Esmaeil Mortaz[4]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Tuberculosis and Lung Disease Research Center of Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The importance of toll-like receptors (TLRs) and their signaling in immune responses against viral infection has been described, however, the role of TLRs in SARS-CoV-2 infection is not well elucidated. It has been confirmed that impaired production of interferons (IFNs), as products of TLRs pathways, is a hallmark of severe and critical COVID-19. Thus, we aimed to investigate the intracellular levels of IFN-β and IFN-γ in activated immune cells, when exposed to the TLRs agonist and SARS-COV-2 antigens (Spike protein (SP) and its receptor binding domain (RBD)).

Methods: Peripheral blood mononuclear cells (PBMCs) of 30 COVID-19 patients (15 severe and 15 moderate) and 10 healthy, age and sex-matched control (HC) were isolated and then activated with agonists of TLR3, 7, 8, 9, SP, and RBD. Frequencies of CD3+IFN-β+ T cells, CD3+IFN-γ+T cells, and gene expression of IFN-βwere evaluated by flow cytometry and qRT-PCR, respectively.

Results: The highest increase of the frequency of CD3+IFN-β+T cells in moderate patients, was seen with TLR8 agonist and SP, which was significantly higher than in severe patients (p=0.0009 and 0.04, respectively) and HC (p <0.0001 and p<0.0001). In the severe group, the highest increase in the frequency of CD3+IFN-β+ T cells was seen with TLR8 and TLR7 agonists. The frequency of CD3+IFN-γ+T cells was significantly increased upon stimulation with TLR agonists in moderate and severe groups, compared with HC, except with TLR7 agonists. The expression of the IFN-βgene after stimulation of isolated CD3+T cells with TLR8 agonist and SP was also up-regulated in moderate than severe patients.

Conclusion: Stimulation of PBMCs of COVID-19 patients with TLR8 agonist and SP upregulated protein and gene expression of IFN-βin T cells and may potentiate the immune responses against SARS-CoV-2 infection and prevent viral replication and spread.

Keywords: "COVID-19", "SARS-CoV-2", "TLR", "IFN-β'

Investigation of Seroconversion Pattern of Specific Antibodies to Different Antigens of SARS-CoV-2 in Hospitalized COVID-19 Patients and Vaccinated Persons: Future Cohort

Shahrzad Matinfar[1], Sahar Mortezagholi[2], Darya Amiri[1], Hossein Pashaee[3], Maryam Eskandarian[1], Somayeh Ghadimi[4], Mohammad Farzad Nazari[4], Shole Tavakoli[4], Melika Valizadeh[4], Saaed Namaki[4], Payam Tabarsi[4], Mehdi Boutrabi[1], Mahdi Shabani[2]

1. Synapse IVD accelerator, Tehran, Iran
2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Hematology, Oncology and Stem cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
4. Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: Induction of humoral response to SARS-CoV-2 could partially control virus dissemination. There is no consistency in the reported kinetics of IgM and IgG to SARS-CoV-2. In addition, the humoral response to SARS-CoV-2 infection could be different from that provoked by vaccination. Thus, we were persuaded to evaluate the kinetics of antibodies against SARS-CoV-2 in infected and vaccinated persons to clarify the serological profile of humoral response to SARS-CoV-2.

Methods: In this cohort study, serial blood and swab samples were collected from 134 COVID-19 patients at six-time points after admission. Real-time RT-PCR specific to SARS-CoV-2 was performed on the samples and anti-SARS-CoV-2 IgM and IgG were checked using ELISA. Furthermore, 141 serum samples were taken from vaccinated persons after the injection of different vaccines. Anti-SARS-CoV-2 spike and RBD IgGs along with neutralizing antibodies in vaccinated and 96 COVID-19 patients were checked using ELISA.

Results: Anti-SARS-CoV-2 IgM was positive in 23.3% of patients at 0-7 days and then seropositivity reached 71.7% at 15-21 days after symptom onset. In the following time points, the IgM positivity gradually decreased to 62.7% at >28 days after symptoms onset. While anti-SARS-CoV-2 IgG was positive in 28.3% and 83.7% of patients at 0-7 and 22-28 days after symptoms onset, respectively, and stayed constant after that. Anti-spike and -RBD IgGs and neutralizing antibodies were detected in 89.7%, 87.4%, and 87.9% of vaccinated and 37.5% and 32.3% and 32.3% of COVID-19 patients, respectively. There was a significant correlation between anti-spike IgG and anti-RBD IgG concentrations in the COVID-19 infected (r=0.801) and vaccinated individuals (r=0.827). Similarly, this correlation was also found between SARS-CoV-2 neutralizing antibodies and anti-spike IgG or anti-RBD IgG. The mean concentration of anti-spike and -RBD IgGs were higher in vaccinated persons who had COVID-19 infection history compared to subjects with no previous infection. Interestingly, 94.1% of seronegative vaccinated persons for neutralizing antibodies had no previous COVID-19 infection.

Conclusion: The antibody profile of IgM and IgG to SARS-CoV-2 indicated that passing time after disease symptoms onset increased the seropositivity of COVID-19 patients. Antibodies to SARS-CoV-2 are produced more efficiently in COVID-19 vaccination compared to natural infection.

Keywords: SARS-CoV-2, COVID-19, cohort, seroconversion, antibody, RT-PCR

Investigation of specific antibody titer against SARS-CoV-2 virus during hospitalization and one month after discharge in hospitalized children with severe and critical Covid-19 infection

Hamidreza Houshmand[1], Reza Jafary[2]

1. Assistant Professor of Allergy and Clinical Immunology, Urmia University of Medical Sciences, Urmia, Iran
2. Assistant Professor of Medical Immunology, Cellular and Molecular Research Center, Cellular and Molecular Medicine Research

Background: The response of the human immune system to the COVID-19 disease is of vital importance in many ways, including determining the place of serological methods in the survival of patients. Therefore, the present study was designed and implemented to determine the specific antibody titer against SARS CoV-2 during hospitalization and one month after discharge in children hospitalized in Shahid Motahari Hospital in Urmia.

Methods: In this longitudinal study, hospitalized patients with positive RT-PCR tests were included in the study due to COVID-19. Inclusion criteria included age less than 15 years and parental consent, and exclusion criteria included immune system disorder or developmental disorder and negative RT-PCR test. Demographic information and severity of the disease along with specific antibody titer of SARS-CoV-2 were evaluated and extracted for all patients.

Results: The average age of the 40 studied patients was 2.48 years, and 32.5% of them were girls. The average level of IgG during discharge and one month after discharge was equal to 73.66 and 128.36, respectively, and it was significantly higher one month after discharge than during discharge (p<0.001). There was no statistically significant difference between the two sexes in terms of antibody titer during discharge (p=0.77) and one month after discharge (p=0.31). There is no significant relationship between the specific antibody level of SARS-CoV-2 during discharge (r=0.12 and p=0.45) and one month after discharge and the length of hospitalization. There was no statistically significant difference between disease severity and antibody titer during discharge (p=0.54) and one month after discharge (p=0.20).

Conclusion: The specific antibody titer of SARS-CoV-2 increases significantly after one month of discharge. There was no significant difference between the level of this antibody during discharge and one month later between girls and boys and people with different severity of the disease. No significant relationship between antibody titer and hospitalization time was observed.

Keywords: Keywords: Children, COVID-19, Hospitalized patients, Specific antibody

Longitudinal study of IFN-αin patients with COVID-19

Sara Ghaffarpour[1], Ali Khodadadi[2], Mahmoud Mahmoudi[3], Rezvan Norouzirad[4], Azadeh Rashidi[1], Mohammad Mehdi Naghizadeh[5], Tooba Ghazanfari[1]

1. Immunoregulation Research Center, Shahed University, Tehran, Iran
2. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Immunology, Shahed University, Tehran, Iran
5. Non-communicable Diseases Research Center, Fasa University of Medical Science, Fasa, Iran

Background: Type I IFNs have critical roles in the defense against viral infection. However, there are conflicting results about the protective or pathologic role of IFN-α Most of IFN-α levels in COVID-19 patients were undetectable. Therefore, a large number of samples are required to obtain reliable results.

Methods: 456 patients with confirmed COVID-19 and 129 sex and aged-matched healthy control were enrolled. The patient with COVID-19 was classified based on hospitalization, requiring ICU and intubation, and mortality. The serum levels of IFN-αwere measured in the first, third, seventh, and fourteen days after admission, using ELISA. The statistical analysis was done using the Mann Whitney test.

Results: At admission, the serum levels of IFN-α declined in outpatients and inpatients (both medians=0.00) in comparison with a control group (median= 8.04, p<0.001, and p=0.002, respectively). However, IFN-αlevels were elevated in intubated patients (median=4.08) compared with outpatients and inpatients (P=0.003 and p=0.008, respectively) at admission. In addition, IFN-α levels were higher in deceased rather than survival patients on the first day (2.79 vs 0.00, p=0.051) and the seventh fourteen days after admission (9.07 vs 0.00, p=0.007). The comparison between each group in the other times did not have significant differences.

Conclusion: Our findings suggest that elevation of IFN-α outcomes severity and mortality in COVID-19.

Keywords: COVID-19, SARS-CoV-2, IFN-α, innate immunity

Misophonia in a young woman infected with COVID-19 omicron variant with a history of asthma: A case report

Mahdi Asghari Ozma[1], Fatemeh Aghamohammadzadeh[2], Manouchehr Fadaee[3], Mahin Ahangar Oskouee[1]

1. Department of Microbiology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Medical student, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: The novel coronavirus known as COVID-19, a communicable infection, surrounded the world, involved many people, and caused a severe global health crisis. This infection can involve various organs of the body and may appear in varied sorts of specimens such as respiratory secretions, urine, feces, blood, semen, and sputum. Patients with this infection show a variety of symptoms such as fever, headache, dry cough, sore throat, fatigue, dyspnea, and loss of smell and taste, which are the foremost common symptoms seen in involved people.

Methods: In one of the patients with COVID-19, who also had a history of asthma, a hospitalized young woman with 70% involvement of the lungs due to COVID-19, and with an increased in white blood cell count, in addition to the common symptoms, she had misophonia, which was a rare symptom and made the patient hatred of surrounding sounds.

Results: In all these and other studies by researchers around the world, there are no significant reports of the patient's sensitivity to sounds like misophonia, and this symptom can help physicians in diagnosing the disease for rapid initiation of treatment and effective infection control. In this case, all of the symptoms of the patient gradually improved and she regained her health after long care in the hospital.

Conclusion: In the diagnosis of patients suffering from various infections, one should pay attention to all the symptoms from the past and present history of the diseases, in order to be effective in the quick and correct diagnosis and the quick initiation of treatment.

Keywords: COVID19, Symptom, Misophonia, Asthma, Pandemia

MMP9 rs3918242 polymorphisms increases the risk of neurological symptoms in the COVID-19 patients

Hamed Mohammadi[1]

1. Alborz University of Medical Sciences, Alborz, Iran.

Background: Recent studies declared that the matrix metalloproteinases-9 (MMP-9) gene was increased in Coronavirus disease 2019 (COVID-19) patients. In this study, we intended to assess the relation of MMP9 rs3918242 polymorphisms with the risk of COVID-19 with the neurological syndrome (NS).

Methods: We obtained 10 ml of peripheral blood from 250 COVID-19 individuals and 250 healthy subjects. MMP9 rs3918242 polymorphisms genotyped by Real-time allelic discrimination method. Enzyme-linked immunosorbent assay (ELISA) was used to detect the level of MMP-9 in serum.

Results: The MMP9 gene rs3918242 SNP was significantly related to enhanced COVID-19 risk and susceptibility to COVID-19 with NS. The MMP-9 serum level was significantly higher in COVID-19 individuals in comparison to controls (p= 0.009). The MMP-9 Serum level was also significantly higher in COVID-19 subjects with NS compared with the controls (p= 0.0003).

Conclusions: MMP9 gene polymorphism promotes susceptibility to COVID-19 and also COVID-19 with the neurologic syndrome.

Keywords: Coronavirus disease 2019, Matrix metalloproteinases-9, Genetic polymorphism, Neurological symptoms

Mucormycosis infection in severe COVID-19 patient with multiple underlying health conditions

Seyedeh Reihaneh Hashemi[1], Zahra Heidarifard[2]

1. Student research committee, Iran.
2. Department of Virology, Iran.

Background: SARS-CoV-2 can cause severe infections in patients who have underlying health conditions. It was suggested that SARS-CoV-2 might increase the susceptibility to pulmonary fungal co-infections. Mucormycosis is an angioinvasive disease caused by a fungus of the order Mucorales. It is a rare infection with a high mortality rate. Immunocompromised patients are susceptible to the highly fatal mucormycosis infection. We present a case of severe COVID-19 with multiple underlying health conditions that were co-infected with rhino-orbital mucormycosis.

Case presentation: A 62-year-old man with a past medical history of type 2 diabetes mellitus, hypertension, and diabetic nephropathy under hemodialysis. He had a history of coronary artery bypass grafting (CABG) and cataract surgery for both eyes. He was diagnosed with acute respiratory distress syndrome (ARDS); therefore, he was admitted to the intensive care unit (ICU).

Conclusion: We believe that uncontrolled diabetes milieus made the patient susceptible to both severe COVID-19 and mucormycosis. Prevention and management guidelines as well as prophylactic treatment protocols are needed for similar complicated patients

Keywords: mucormycosis, SARS-CoV-2, severe COVID-19, underlying health conditions

Multi-targeted therapeutic potential of genus Salix aegyptiaca extract against COVID-19

Zahra Azadian[1], Meysam Soleimani[2]

1. Research center for Molecular Medicine, Hamadan University of Medical Sciences, and Hamadan, Iran.
2. Department of Pharmaceutical Biotechnology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

Background: The COVID-19 pandemic is a contagious respiratory disease that results in severe illnesses such as pneumonia, acute respiratory distress syndrome (ARDS), and hyperinflammatory manifestations. Interventions that can be given early during infection, such as antiviral drugs are urgently needed to stop the progression of the disease and long-term complications. Immune dysregulation is also an essential element of the pathophysiology of COVID-19. Therefore, in the treatment options for critically ill patients requiring late-stage hospitalization, the utility of immune-based therapies to improve outcomes in patients with COVID-19 is under intense investigation. In such cases, reusing multi-target therapies is considered the best approach for systemic diseases similar to COVID-19. During the COVID-19 pandemic, it was crucial to focus drug research on testing new herbal medicines to gain access to effective targeted therapies, as well as antiviral and anti-inflammatory drugs.

Methods: In the present study, XP Glide docking with Schrödinger-Maestro software was used to assess the possible inhibitory effects of bioactive compounds from Salix extract on angiotensin-converting enzyme-2 (ACE-2), 3C-like protease (3CLpro), and RNA-dependent RNA polymerase (RdRp). The most active compounds in the Salix extract were docked to the active site residues of target receptors compared to known inhibitors such as Remdesivir, Lopinavir/ritonavir, and Chloroquine. As part of the study, we also determined the precise molecular mechanisms of the active ingredients of Salix by network pharmacology approach. The network was obtained using the STRING and STITCH databases and therefore analyzed with the Cytoscape network analysis tool.

Results: Molecular docking results showed that molecular binding affinity was lower for Remdesivir (-6,696 kcal/mol), Lopinavir/ritonavir (-7,211 kcal/mol), and Chloroquine (-5.694 kcal/mol) compared to rutin, myricetin, quercetin, catechin, and salicin, Suggesting the potential of numerous active compounds in Salix to interact directly with ACE-2, 3CLpro, and RdRp. Network pharmacology research showed that Salix extract could decrease PTGS2 receptor expression, followed by alleviating cytokine storm, resulting in anti-inflammatory effects.

Conclusion: In the study, our in-silico data suggests that Salix extracts may demonstrate anti-viral and anti-inflammatory effects with significant power in the fight against Covid-19; due to its high binding affinity to related receptors. The testing phase is underway to evaluate the effectiveness of Salix extract in SARS-CoV-2 infection.

Keywords: COVID-19, molecular docking, Salix aegyptiaca, multi-target therapy

NLRP3 gene expression in PBMCs of COVID-19 patients and their relationship to disease severity

Alireza Andalib[1], Maedeh Radandish[1], Nafiseh Esmaeil[1], Farzin Khorvash[2]

1. Immunology department, Isfahan Medical School-Isfahan University of Medical Sciences, Isfahan, Iran.
2. Infectious disease department, Isfahan Medical School-Isfahan University of Medical Sciences, Isfahan, Iran.

Background: NLRP3 (NLR family pyrin domain containing 3) is an intracellular sensor that detects a variety of endogenous danger signals, resulting in the formation and activation of the NLRP3 inflammasome. NLRP3 is expressed predominantly in macrophages. Activated NLRP3 in turn triggers an immune response that leads to caspase 1-dependent release of the pro-inflammatory cytokines such as IL-1β and IL-18. NLRP3 is a component of the innate immune system that functions as a pattern recognition receptor (PRR) that recognizes pathogen-associated molecular patterns (PAMPs). NLRP3 inflammasome complex activates inflammatory cytokines which are critical for disease progression in SARS-CoV-2 infection. So, the study was planned to measure NLRP3 gene expression in COVID-19 patients for evaluation of the severity of the disease's clinical situation and its relationship with other para-clinical parameters.

Methods: RNA was extracted from peripheral blood according to the kit instruction, from 20 healthy individuals and 44 COVID-19 patients. cDNA synthesis was performed, and NLRP3 and GAPDH genes were evaluated using a Real-time PCR technique. Allele ID 7.0 software was used to design specific primers and the BLAST program was applied in order to ensure the specific binding of primers on the genome.

Results: NLRP3 gene expression was calculated at 1.90±0.32 in the control group and 9.71±1.88 in COVID-19 patient samples (p=0.0013). In addition, the quantity of NLRP3 gene expression was 8.64±1.71 in the severe situation of disease and 13.98±6.60 in the critical situation of patients. The association of lung involvement and other para-clinical inflammation factors such as D-Dimer, LDH, and CRP quantity were recorded for comparison their association.

Conclusions: enhanced NLRP3 gene expression in COVID-19 patients was associated with disease severity and other clinical inflammatory factors. NLRP3 expression could be used as meaningful indicators for assisting the diagnosis of severe/critical COVID-19. It could be speculated that control of the NLRP3 expression in the immune cells may help inflammatory cytokine storm.

Keywords: COVID-19, NLRP3, inflammasome

Potential Adjuvant Effect of Lactobacillus acidophilus for treatment of SARS-CoV-2

Maryam Mousavi[1], Monireh Kamali[2], Sheida Janati[3], Hale Radi[4], Sarah Shahabi Aghdam[5]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Rajaei Cardiovascular Medical Center, Iran University of Medical Sciences, Tehran, Iran
3. Faculty of Modern Biological Sciences and Technologies, University of Science and Culture, Tehran, Iran
4. Department of Medical Microbiology and Biotechnology, Faculty of medicine, Babol University of Medical Sciences, Babol, Iran
5. Students Research Committee, Faculty of medicine, Ardabil University of Medical Sciences, Ardabil, Iran

Background: Given the ongoing COVID-19 pandemic caused by SARS-CoV-2, there is an urgent need for exploring potential therapeutic interventions. Lactobacillus acidophilus is a well-studied probiotic with a range of health benefits. The potential adjuvant effect of L. acidophilus for the treatment of SARS-CoV-2 was investigated in this study.

Methods: The study was carried out in vitro using cell cultures infected with SARS-CoV-2. The effects of treatment with L. acidophilus on virus replication and cell viability were evaluated. The levels of pro-inflammatory cytokines were also measured to assess the potential anti-inflammatory effects of L. acidophilus treatment.

Results: Treatment with L. acidophilus showed a significant reduction in SARS-CoV-2 viral replication. The levels of pro-inflammatory cytokines were also significantly reduced in cells treated with L. acidophilus. Additionally, L. acidophilus did not affect the viability of the infected cells.

Conclusions: The findings of this study suggest that L. acidophilus treatment may have a potential adjuvant effect of treatment SARS-CoV-2. The reduction in viral replication and pro-inflammatory cytokine levels without affecting cell viability highlights its potential as a safe and effective therapeutic strategy.

Keywords: Adjuvant, Lactobacillus acidophilus, SARS-CoV-2

Preclinical assessment of a recombinant RBD-Fc fusion protein as SARS-CoV-2 candidate vaccine

Navid Dashti[1], Forough Golsaz-Shirazi[1], Mohammad Mehdi Amiri[1], Haleh Soltanghoraee[2], Amir-Hassan Zarnani[1], Mehdi Mohammadi[3], Mahmood Jeddi-Tehrani[2], Fazel Shokri[1]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
2. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
3. Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran.

Background: Safe and efficient vaccines are essential to control the COVID-19 pandemic. The spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) consists of a receptor-binding domain (RBD) that binds to the angiotensin-converting enzyme 2 (ACE2), the virus receptor on host cells. RBD contains numerous dominant neutralizing epitopes and is the main target for COVID-19 vaccine development.

Methods: A recombinant fusion protein containing RBD of SARS-CoV-2 with a human IgG1 Fc fragment, as an immunopotentiator, (designated RBD-Fc) was produced in a mammalian CHO expression system. The safety and immunogenicity of the candidate vaccine were studied in mice and rabbits.

Results: The RBD-Fc fusion protein emulsified in Alum adjuvant induced high titer of anti-RBD antibody with potent neutralizing activity in the immunized mice and rabbits. The antisera of mouse and rabbit recognized RBD and effectively inhibited the binding of RBD to ACE2. The antisera could also effectively neutralize infection by pseudotyped SARS-CoV-2. The fusion protein induced a significant IFN-γ and IL-13, but not IL-17 and TGF-β cytokine response in immunized mice. Toxicity studies showed no adverse events. Histopathologic examination demonstrated no substantial pathologic changes in different organs such as the brain, heart, lung, thymus, liver, and inguinal lymph node. No changes in serum biochemical parameters and peripheral blood differential cell counts were observed.

Conclusion: These results suggest that the recombinant CHO-expressed RBD-Fc fusion protein can elicit highly robust neutralizing antibody and TH1 responses with no adverse events and has the potential to be further developed as an efficient and safe subunit vaccine for the prevention of current SARS-CoV-2 infection.

Keywords: SARS-CoV-2, Vaccine, RBD-Fc, Immunogenicity, Toxicity

Production of the Neutralizing monoclonal antibody against RBD protein of SARS-CoV-2

Seyed Mostafa Mostafavi Zadeh[1]

1. Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran.

Background: Humoral immunity plays a critical role in defense against SARS-CoV-2 infection. To stop the spread of the COVID-19 disease, it is crucial to create molecular tools for investigation, diagnosis, and treatment of COVID-19.

Methods: Current efforts focus on developing specific antiviral drugs such as Neutralizing monoclonal antibodies (NmAbs) which are elicited against parts of the spike protein such as the receptor-binding domain (RBD) as a major target for the antibody response. In the present study, mice were immunized with recombinant RBD which corresponds to engaging the host receptor ACE2. After the selection of immunized mice, hybridomas were produced by standard protocol, and 5 RBD-specific clones were screened. Then purified mAbs were able to detect specifically commercial and denatured RBD proteins and the reactivity of the final 5 clones was assessed by applying enzyme-linked immunosorbent (ELISA).

Results: We showed that 2 out of 5 mAbs could neutralize RBD by competing with ACE2.

Conclusion: These observations suggest that the production of neutralizing mAbs against the RBD protein is critical for the development of diagnostic techniques and its humanized mAbs have therapeutic applications.

Keywords: COVID-19, Monoclonal antibody, SARS-CoV-2, ACE-2

Profiling serum levels of glutathione reductase and interleukin-10 in positive and negative-PCR COVID-19 outpatients: A comparative study from southwestern Iran

Sahar Golabi[1], Mahshid Naghashpour[1], Hamid Ghiassian[1], Sara Mobarak[1], Maryam Adelipour[1], Maghsud Piri[1], Maryam Seyedtabib[1]

1. Abadan University of Medical Sciences, Abadan, Iran.

Background: Since the outbreak of COVID-19 in China, it has rapidly spread across many other countries. We evaluated antioxidant defense systems and inflammatory status related to the SARS-CoV2 infection in a population from southwestern Iran.

Methods: Comorbidities and clinical symptoms of 104 subjects (comprising negative and positive-PCR COVID-19 outpatients) were assessed. Serum concentrations of glutathione reductase (GR) and interleukin-10 (IL-10) were measured using ELISA. In the positive-PCR group, follow-upson clinical symptoms were carried out for 28 days at 7-day intervals. In the positive-PCR group, hypertension, diabetes, liver disease, chronic heart disease, and chronic kidney disease were the most common comorbidities.

Results: In the general category of symptoms, we found a significant difference between negative and positive-PCR groups, except regarding runny noses. In the pulmonary category, there was a significant difference between the two groups except in terms of chest pain. We also determined a significant difference in neurologic symptoms, except for ear pain, between negative and positive-PCR groups. We also found significantly lower levels of GR but higher levels of IL-10 in the positive-PCR group ($p = 0.0001$ for both). In the positive-PCR group, serum levels of IL-10 (odds ratio = 0.914, $p = 0.012$) decreased the chances of neurological symptoms occurring over time.

Conclusion: The antioxidant defense systems of positive-PCR outpatients failed as demonstrated by a reduction in the serum levels of GR. We also indicated a dysregulation in the immune response against COVID-19, characterized by changes in serum IL-10 levels.

Keywords: COVID-19, glutathione reductase, interleukin-10, southwestern Iran

Relationship between complement system in COVID-19 patients at the time of admission and the clinical outcomes

Hasan Namdar Ahmadabad[1], Maryam Nezafat Firizi[2]

1. Department of Pathobiology and Laboratory Sciences, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.
2. Department of Pathobiology and Laboratory Sciences, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.

Background: Although several studies have been carried out on the relationship between complement systems in COVID-19 and clinical outcomes, they did not indicate a clear protective or adverse effect of it. Therefore, we conducted a study to assess the association between complement system status at the time of admission and clinical outcomes in COVID-19 patients.

Methods: This case-control study was performed on sixty-one COVID-19 patients who were hospitalized at Imam Hassan Hospital (Bojnourd, Iran). Twenty-three healthy volunteers were included as the control group. Patients' information including demographic information, demographic data, clinical characteristics, and clinical outcomes obtained from electronic medical records. The levels of C3, C4, and CH50 were determined through the immunoturbidimetric method and single-radial-hemolysis plates, respectively, on serum samples obtained from patients at the time of admission and the control group.

Results: Our results indicate that levels of C3, C4, and CH50 are markedly lower in COVID-19 patients than in the control group. We also found that levels of these values of complement parameters in COVID-19 patients who died or were admitted to the ICU were significantly lower than in COVID-19 patients who did not admit to ICU.

Conclusions: In general, it seems that serum levels of C3, C4, and CH50 at admission may predict disease progression and adverse clinical outcomes in COVID-19 patients.

Keywords: COVID-19; Complement System; Clinical outcome

Serological diagnosis of SARS-CoV-2 by novel nucleocapsid specific monoclonal antibodies using a sandwich ELISA

Hamidreza Yadegari[1], Fazel Shokri[1], Mehdi Mohammadi[1], Faezeh Maghsood[1], Ahmad Ghorbani[1], Tannaz Bahadori[1], Amir-Hassan Zarnani[1], Vahid Salimi[2], Mahmood Jeddi-Tehrani[3], Mohammad Mehdi Amiri[1]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Monoclonal Antibody Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran

Background: The current coronavirus disease 2019 (COVID-19) pandemic is a devastating threat to health, economy, and society worldwide. Early virus detection is essential for disease control and management. The gold standard detection method, quantitative real-time PCR (RT-qPCR), has several limitations. Viral antigen detection by ELISA, as a cost-effective, rapid, and accurate antigen diagnostic assay, may facilitate pandemic management and public health improvement.

Methods: We developed an antigen-capture sandwich ELISA using nucleocapsid protein (NP)-specific mouse monoclonal antibodies (MAbs). A total of 553 respiratory samples including 403 positive and 150 negative samples, which had been collected during different SARS-CoV-2 variants outbreaks in Iran, were analyzed to assess the diagnostic performance of the assay.

Results: The limit of detection of our ELISA assay was found to be 43.3 pg/ml for recombinant NP. The overall sensitivity and specificity of this assay were 70.72% (95% CI: 66.01-75.12) and 100% (95% CI: 97.57-100), respectively, regardless of cycle threshold (Ct) values and SARS-CoV-2 variants. There was no significant difference in our assay sensitivity for the detection of Omicron subvariants compared to the Delta variant. Assay sensitivity for the BA.5 Omicron subvariant was calculated as 91.89% (95% CI: 85.17-96.23) for samples with Ct values < 25 and 82.70% (95% CI: 75.19-88.71) for samples with Ct values < 30.

Conclusion: Our newly developed ELISA method is reasonably sensitive and highly specific for the detection of SARS-CoV-2 regardless of the variants and subvariants of the virus.

Keywords: ELISA, Monoclonal antibody, Nucleocapsid, SARS-CoV-2, COVID-19

Serum trace elements levels and their correlation with severity and outcomes in COVID-19 patients

Roya Mahdavi[1], Nooshin Ghadiri[1], Zahra Akbari Jonoush[1]

1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Background: SARS-CoV-2 is a novel coronavirus that has caused many deaths in the recent pandemic. It is well known that nutritional deficiency is associated with impaired immunity and increased susceptibility to infections. Hence, we aimed to evaluate the association of serum levels of trace elements with severity and survival status in COVID-19 patients.

Methods: In this case–control study, a total of 80 participants (45 men and 35 women; age range, 20–75 years; average age, 53.2 years) were admitted to severe intensive care units (ICUs), and 80 healthy individuals with age and sex-matched were involved as the control group. Serum levels of copper, zinc, manganese, and magnesium were determined by atomic absorption spectrometry.

Results: At the time of ICU admission, Serum concentration of copper, zinc, manganese, and magnesium were measured. Serum concentrations of copper ($p < 0.001$) and zinc ($p < 0.001$) were lower and manganese ($p < 0.01$) was higher in patients compared to healthy controls. On the other hand, no significant differences were observed between patients and controls in the serum levels of magnesium. Increased serum levels of copper and zinc concentrations during ICU care were associated with significantly lower death among COVID-19 patients (p <0.005), but manganese serum levels were not associated with patient outcomes.

Conclusion: These results suggest that decreased serum zinc and copper levels may be a risk factor for COVID-19 infection, so they can be considered for monitoring patients' prognosis. Therefore, supplements can help reduce the poor outcomes caused by low levels of zinc and copper in COVID-19 patients.

Keywords: COVID-19, Trace elements, Zinc, Copper, Magnesium, Manganese

Side effects of the COVID-19 vaccines in children aged 5-12 years in Iran, North Khorasan

Maryam Nezafat Firizi[1], Hasan Namdar Ahmadabad[1], Shahin Mafinezhad[2]

1. Department of Pathobiology and Laboratory Sciences, North Khorasan University of Medical Sciences, Bojnurd, Iran
2. Department of Pediatrics, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

Background: Vaccination of children against COVID-19 in Iran has conducted for children 5-12 years on July 22, 2021, with PastoCovac and Sinopharm BIBP. This study aimed to evaluate the side effects following the administration of vaccines in children.

Methods: This descriptive-analytical study was performed on 394 children aged 5-12 years who were referred to health centers in Bojnurd, Iran, and received PastoCoVac or Sinopharm BIBP COVID-19 vaccines from March to July 2022. After receiving the first dose of the vaccine, the children's parents filled out sections of a COVID-19 vaccine survey questionnaire that included sociodemographic and clinical characteristics of children in health centers. Thereafter, other sections of the survey questionnaire related to side effects following vaccination were filled out by parents of children at different time intervals after receiving the first or second dose of vaccines.

Results: Our results showed that the incidence of vaccine side effects in children aged 5-12 years varies between 24%-37%, depending on the type and dose of vaccine. The most common side effects after getting the first and second doses of vaccines were injection site pain and swelling, fever, fatigue, and myalgia. No serious side effects were reported and almost side effects were resolved within a few days without special treatment.

Conclusion: Altogether, these results suggest the safety of PastoCoVac and Sinopharm BIBP vaccines for children aged 5-12 years is acceptable. The most common side effects post-vaccination was mild and transient. Therefore, it could be concluded that the high-quality and safe results reported here could increase parents' desire for children's COVID-19 vaccination in Iran.

Keywords: Side effect, injections, fever, fatigue, myalgia.

Simultaneous enhancement of TNF-αand IFN-γlead to inflammation, severity and mortality in COVID-19

Sara Ghaffarpour[1], Ali Mohammad Mohseni Majd[1], Hossein Ali Khazaei[2], Abbas Rezaei[3], Amrollah Mostafazadeh[4], Fatemeh Tuserkani[1], Tooba Ghazanfari[1]

1. Immunoregulation Research Center, Shahed University, Tehran, Iran.
2. Department of Immunology and Internal Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.
3. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
4. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.

Background: SARS-CoV-2 infection results in a broad spectrum of disease severity. Unregulated elevation of circulating cytokines occurs during SARS-CoV-2 infection. There is a report that the synergism of TNF-αand IFN-γcause a life-threatening cytokine shock in mice that is similar to the tissue damage and inflammation of COVID-19. However, there is no tangible evidence of whether simultaneous elevation of these cytokines induced in COVID-19 triggers severity and mortality.

Methods: Eight hundred and fifty-seven patients with confirmed COVID-19 and sixty sex and aged-matched healthy control were enrolled. The serum levels of TNF-α IL-6, and IFN-γ were measured using ELISA. The participants were classified based on the levels of TNF-αand IFN-γ: patients with simultaneously increased levels of TNF-αand IFN-γ(Group 1), patients with high levels of TNF-αand low levels of IFN-γ(Group 2), patients with low levels of TNF-αand high levels of IFN-γ(Group 3), and patients with simultaneous low levels of TNF-αand IFN-γ(Group 4).

Results: the frequency of patients in Group 1 was approximately double in inpatients versus outpatients (p<0.001), in patients requiring ICU versus non-ICU (p<0.001), intubated versus non-intubated (p=0.044), and survival versus non-survival patients (p=0.031) in compared with patients Group 2. In addition, serum levels of IL-6 and ferritin were dramatically higher in patients in Group 1 compared with patients in Group 2, patients in Group 3, and patients in Group 4 (all p<0.05). However, CRP levels were significantly higher in Groups 1,2, and 3 in comparison with patients in Group 4.

Conclusion: Our findings suggest that the evaluation of simultaneous changes of TNF-αand IFN-γcould help us to identify dysregulated inflammatory mechanisms in COVID-19.

Keywords: COVID-19, TNF-α IFN-γ, inflammation.

Study of frequency and inheritance model of ACE1 I/D and ACE2 rs2285666 polymorphisms in COVID-19 patients with varying severity of lung involvement and its effect on serum cytokines levels

Shirin Beyranvand[1], Parivash Davoodian[2], Hesam Alizade[2], Hamed Gouklani Gouklani[2], Azim Nejatizadeh[3], Ebrahim Eftekhar[4], Amin Reza Nikpoor[4]

1. Student Research Committee, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
2. Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
3. Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
4. Endocrinology and Metabolism Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Background: The angiotensin-converting enzyme (ACE) has been shown to play a role as a receptor for the COVID-19 virus. This virus usually gets into cells and infects them by attaching to their glycoprotein receptors, which are found on the ACE2 receptor. This study aimed to evaluate the frequency and inheritance of ACE1 I/D and ACE2 rs2285666 polymorphisms in COVID-19 patients with varying severity of lung involvement and its effect on serum cytokines levels of interleukin (IL)-1 and IL-6 and laboratory parameters.

Methods: One hundred eighty-five COVID-19 patients were grouped according to the severity of lung involvement. (I/D) polymorphism of the ACE1 gene and rs2285666 polymorphism of the ACE2 gene were determined by a single specific primer-polymerase chain reaction and restriction fragment length reaction-polymerase chain reaction methods, respectively. Serum levels of IL-1 and IL-6 were also measured by the enzyme-linked immunosorbent assay technique.

Results: No statistically significant association of ACE2 rs2285666 polymorphism genotypes and ACE1 I/D with the severity of lung involvement was noted. However, there was a statistically significant association between I/D ACE1 polymorphism genotypes and IL-6, white blood cells (WBC), and neutrophil-to-lymphocyte ratio (NLR) levels. Also, there was no statistically significant association between rs2285666 polymorphism genotypes and patients' blood oxygen saturation level, IL-6, IL-1β lactate dehydrogenase activity, WBC count, and NLR.

Conclusion: In patients with COVID-19, the rs2285666 polymorphism of the ACE2 gene and the I/D polymorphism of the ACE1 gene were not significantly associated with the severity of COVID-19 disease and serum IL-6 and IL-1 cytokine levels.

Keywords: ACE1 protein, ACE2 protein, COVID-19, single nucleotide polymorphisms

Study on the association between IL-6R complex expression and interleukin-6 receptor Asp358Ala (rs2228145) single nucleotide polymorphism, with the severity of clinical symptoms in patients with COVID-19

Azadeh Rashidi[1], Tooba Ghazanfari[1]

1. Immunoregulation Research Center, Shahed University, Tehran, Iran.

Background: Interleukin 6 (IL-6), a pro-inflammatory and anti-inflammatory cytokine, is known as a major component in regulating a wide variety of physiologic and pathological processes including COVID-19. IL-6 exerts its pro-inflammatory properties through trans-signaling, while mediates its anti-inflammatory activity through classic signaling. Furthermore, genetic variants of the IL-6 receptor including Asp358Ala (rs2228145) are proposed to be involved in the beginning and progression of severe infections such as COVID-19.

Methods: Current study was conducted on 736 patients with COVID-19 and 57 healthy controls. DNA and RNA were extracted from peripheral blood samples of patients with written ethical consent. The IL-6R and sIL-6R mRNA expression levels were assessed using RT-PCR. The specific genotype of rs2228145 polymorphism was assayed by RFLP PCR. Data analysis was performed using SPSS software (version 24).

Results: Based on our data, significant decreases were observed in IL-6R mRNA expression in the inpatient group compared to the outpatient (p<0.001). Moreover, sIL-6R mRNA expression was significantly different in diverse groups: COVID-19>Control (p<0.01), Inpatient>Outpatient (p<0.05); while non-ICU>ICU (p<0.01), non-intubated>intubated (p<0.05), Alive>Expire (p<0.05). Regarding the frequency of rs2228145 variants, no significant difference was observed between COVID-19 and control groups, while in a recessive model of analysis, a significant difference was observed in the CC genotype compared to others in patients who underwent intubation with respect to non-intubated patients.

Conclusion: In conclusion, the abovementioned findings suggest that different levels of IL-6 receptors mRNA expression and related polymorphism rs2228145 could potentially influence the outcome of COVID-19 in the Iranian population.

Keywords: Covid-19, Polymorphism, Interleukin-6 receptor, rs2228145, IL-6R, sIL-6R

T helper subsets concentrations of critical COVID-19 patients after 5 days of treatment with Remdesivir

Maryam Noroozi[1], Atefe Ghamar Talepoor[1], Seppo Meri[2], Kurosh Kalantar[3], Afagh Moattari[4]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Bacteriology and Immunology and the Translational Immunology Research Program (TRIMM), The University of Helsinki and HUSLAB, Helsinki University Hospital, Helsinki, Finland.
3. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
4. Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran.

Background: Today, the world is faced with a new pandemic caused by the emerging Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). One of the main factors that directly correlated with the severe manifestations of covid-19 disease is the cytokine storm. There is no current consensus regarding COVID-19 treatment, But Common treatments known to reduce cytokine storm mainly include the administration of remdesivir, corticosteroids, and tocilizumab. It is vital to check the performance of these drugs in practice. In this study, we intend to evaluate the serum concentrations of different cytokines in the redeliver-treated critical COVID-19 patients in comparison to critical COVID-19 individuals who did not receive redeliver and healthy controls.

Methods: 29 confirmed critical COVID-19 patients in ICU and 29 healthy control were enrolled in this study. Blood was collected from control and patients 5 days before and after treatment. Serum samples were separated using centrifugation. The IL-1 beta, IL-1β, IFN-alpha2, IFN-α2, IFN-gamma, IFN-γ, TNF-α, TNF-alpha, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33 was assessed by multiplex immunoassay method using fluorescence labeled cytokine panel.

Results: The lower serum levels of IL-6 (20.73 pg/mL vs. 134.75 pg/mL, P< 0.0001), TNF-α (10.15 pg/mL vs. 121.67 pg/mL, *p*< 0.0001), and IFN-γ(22.27 pg/mL vs. 29.69 pg/mL, *p*=0.005), as well as a higher serum level of IL-4 (12.44 pg/mL vs. 8.47 pg/mL, P= 0.002), were found in critical COVID-19 patients after treatment with redeliver as compared with those subjects before treatment.

Conclusion: Remdesivir treatment led to decreased levels of pro-inflammatory, Th1, and Th17 cytokines and increased Th2 cytokines in critical COVID-19 patients after 5 days of treatment.

Keywords: COVID-19, SARS-CoV-2, Cytokines storm, Remdesivir, inflammatory cytokines

T lymphocyte Exhaustion and Reduction under Serological Changes, and the Predictors of COVID-19 Progression

Mehdi Yousefi[1], Shabnam Babaei[1], Mohammad Sadegh Soltani Zangbar[1], Amin Kamrani[1], Forough Chakari Khiavi[2], Javad Ahmadian Heris[3], Ata Mahmoodpoor[1]

1. Immunology Department, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Stem Cells Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Allergy and Clinical Immunology, Pediatric Hospital, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Coronavirus disease 2019 (COVID-19) is an almost new viral infection that poses a threat to global health since there is currently no viable treatment plan in place. T lymphocytes are critical components of the body's antiviral defenses. However, the condition may have an impact on T cell frequency and performance.

Methods: Whole blood samples were taken from patients with mild and severe COVID-19. The pro-inflammatory cytokine concentrations in each patient's serum were examined using an enzyme-linked immunosorbent assay (ELISA). Further, the total lymphocyte number, as well as CD4+ and CD8+ T cells, were determined by Flow Cytometry, followed by assessing the expression of markers for exhausted T cells. Finally, the collected results and laboratory serological data underwent analyses.

Results: Patients with severe COVID-19 symptoms had noticeably higher levels of serum tumor necrosis factor-α(TNF- α), interleukin (IL)-1β and IL-2 receptor (IL-2R) than did healthy controls. Patients exhibited Lymphopenia, decreased CD4+ and CD8+ T cells, and a high percentage of Programmed cell death protein-1 (PD-1) expression by T cells, particularly in severe cases. Furthermore, the severity of the condition was associated with high triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels.

Conclusion: Overall, as the illness progressed, pro-inflammatory cytokine release increased as well. A high serum IL-2R level was also observed as a symptom of Lymphopenia. Patients with COVID-19 demonstrated decreased T cell numbers and functionality, particularly in more severe cases. Eventually, hyperlipidemia and hypercholesterolemia could also have a significant predictive role in predicting the severity of the infection.

Keywords: COVID-19, T lymphocyte, Exhaustion, Cytokine

Targeting Citrate Carrier (CIC) in Inflammatory Macrophages as a Novel Metabolic Approach in COVID-19 Patients: A Perspective

Mahsa Eshkevar Vakili[1], Zahra Saleh[1], Dieter Kabelitz[2], Kurosh Kalantar[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Institute of Immunology, University of Kiel, Kiel, Germany.

Background: Coronavirus disease-19 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). SARS-CoV2, an enveloped virus belonging to the Beta coronavirus subfamily, enters the targeted cells, replicates, and causes cellular damage and pulmonary inflammation. Infected lung cells produce large amounts of inflammatory cytokines and chemokines. Then, the recruitment of monocytes and accumulation of inflammatory (M1) macrophages will occur at the site of infection. It seems that a reduction in the activity of M1 macrophages and inflammation has some promising therapeutic effects.

Methods: Therefore, it is plausible that studying the metabolic pathways of these cells during the SARS-CoV2 infection and targeting these pathways can open a new venue to cope with this disease. Studies have shown that the expression of mitochondrial citrate carrier (CIC), the protein that regulates citrate traffic between the cytosol and mitochondria, increases in activated macrophages.

Methods: The elevated CIC level contributes to cytosolic citrate increase and inflammatory mediator production.

Conclusion: Therefore, in this perspective article, we discuss the role of CIC in the metabolism of inflammatory macrophages and propose that inhibition of this carrier can be a novel therapeutic approach for COVID-19 patients.

Keywords: COVID-19, Inflammatory macrophages, Mitochondrial citrate carrier, Cytosolic citrate, Inflammatory mediators.

The Association between Vitamin-D Deficiency and COVID-19 Severity

Nima Zafari[1], Zeinab Faghfoori[2]

1. Student Research Committee, Department of Nutrition, School of Nutrition and Food Sciences, Semnan University of Medical Sciences, Semnan, Iran
2. Ph.D., Food Safety Research Center (salt), Department of Nutrition, School of Nutrition and Food Sciences, Semnan University of Medical Sciences, Semnan, Iran

Background: The coronavirus disease 2019 (COVID-19) outbreak has rapidly expanded into a global pandemic and many aspects of its pathogenesis and related clinical consequences are still unclear. Vitamin D due to its immunomodulatory effect, has been proposed as a factor playing a role in the organism's response to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. This study aimed to evaluate the association between vitamin D deficiency and the severity of COVID-19. Clinical features and infection-related risk factors are also briefly discussed.

Methods: PubMed, Google Scholar, and Web of Science databases were searched for studies evaluating the association between vitamin D deficiency and the severity of COVID-19 infection.

Results: Hypovitaminosis D is attributed to the increased risk of lung injury and acute respiratory distress syndrome (ARDS) as well as diabetes, cardiovascular events, and associated comorbidities, which are the main causes of severe clinical complications in COVID-19 patients. The protective effect of vitamin D is exerted through multiple mechanisms such as modulation of ACE-2 receptor activity, triggering of innate and adaptive immune responses, and reducing the levels of cytokines. Vitamin D maintains intercellular connections and prevents the penetration of viruses into the depths of tissue. Vitamin D helps the immune system by increasing the production of antimicrobial peptides such as cathelicidin and beta-defensin, increasing the phagocytosis of macrophages and stimulating the differentiation of immune cells in the lungs. Also, vitamin D reduces the excessive secretion of inflammatory cytokines and prevents cytokine storms in the lung and other organs. Regulating the renin-angiotensin system and preventing the sharp increase of angiotensin 2 are other functions of vitamin D against the coronavirus. Vitamin D deficiency is significantly associated with high mortality, high hospital admission, longer hospital stay, and ICU admission. Supplementation can reduce the risk of mortality, severity, ICU admission rate, and mechanical ventilation in patients with both mild and severe symptoms.

Conclusion: In general, greater severity of COVID-19 infection can be associated with Vitamin D deficiency. According to the prevalence of vitamin D deficiency in populations, supplementation and controlling serum levels of vitamin D can reduce mortality, especially in high-risk individuals. More studies can help to understand the benefit of vitamin D in COVID-19.

Keywords: Coronavirus, SARS-CoV-2, COVID-19, Vitamin D Deficienc, Severity, Mortality

The effect of 8 weeks of aerobic exercise on the quality of life of people recovered from Covid-19 disease

Soheila Binabaji[1], Mohammad Rahimi[1], Hamid Rajabi[2], Daniel Gahreman[3]

1. Faculty of Physical Education and Sports Science, Kharazmi University, Tehran, Iran
2. Professor of Exercise Physiology, Kharazmi University, Tehran, Iran
3. Charles Darwin University, College of Health and Human Sciences, Northern Territory, Australia

Background: The decrease in the quality of life in Covid-19 patients is visible. Improving this index through aerobic exercise can be promising. The present study aimed to investigate 8 weeks of aerobic exercise on the quality of life of people who recovered from Covid-19 disease.

Methods: 28 people recovered from covid-19 disease with an average age of 32 years and a body mass index of 25.97 kg/m2 were divided into two aerobic training groups (14 people) and a control group (14 people) according to the same method. The subjects of the exercise group performed aerobic exercise 3 sessions a week for 8 weeks for one hour and with moderate intensity. To investigate the quality of life, a questionnaire method was used through the WHOQOL-26 questionnaire, and ANOVA statistical method with repeated measurements was used to analyze the data and a significance level (p <0.05) was considered.

Results: The findings of the present study showed 8 weeks of aerobic training did not make a significant change in the quality of life of people who recovered from the disease of Covid-19. (p<0.05).

Conclusion: Although the level of quality of life improved in the training group compared to the control group after 8 weeks of training, this improvement did not statistically lead to a significant difference between the two groups. This improvement in various components of quality of life including physical and psychological and social, was observed that the changes in the social field were more significant. Considering the positive, albeit relative, effect of aerobic exercise on the quality of life index, it can be very cautiously said that 8 weeks of aerobic exercise improves the quality of life of people recovered from Covid-19 disease through the improvement of the mentioned components.

Keywords: Covid-19, quality of life, aerobic exercise, WHOQOL-26

The effect of remdesivir as an anti-COVID-19 drug on chicken hepatocytes enzymes; an in vitro-study

Roya Mahdavi[1], Maryam Farzaneh[2], Mehri Ghafourian[1], Zahra Akbari Jonoush[1]

1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: There is little evidence of potential hepatotoxicity of Remdesivir (RDV), in vitro. Regarding the effective results of RDV in patients with COVID-19 and the impact of COVID-19 on liver function, we investigated the effects of RDV on the expression and activity of liver enzymes in chicken embryo-derived hepatocytes.

Methods: 20 embryonated chicken eggs (stage X) were incubated (37.5°C, 60-65% humidity) for 10 days (stage HH35). The liver cells were cultured in DMEM/F12+10% FBS medium. After 3 days, four concentrations of RDV (2.00, 3.00, 4.00, and 5.00 µM) were added to the culture medium. Serum levels of alanine (ALT) and aspartate (AST) aminotransferases were measured by Elisa and gene expression was measured by quantitative real-time PCR (qPCR).

Results: Each hepatocyte had a hexagonal structure with a large nucleus and nucleolus. In the PAS (Periodic acid–Schiff) staining, the PAS-positive cells with a pink color confirmed the glycogen content of hepatocytes. In the presence of 4 and 5 µM RDV, up to 50% of hepatocytes lose their viability after 48 hr (p<0.001). Besides, the expression of both ALT and AST was significantly increased after treatment with RDV (p<0.001). Our data showed that the function of both ALT and AST was increased in the RDV+ hepatocytes compared to the RDV- cells (p<0.001).

Conclusion: We concluded that the expression and function of hepatocyte enzymes were increased following treatment with RDV.

Keywords: COVID-19, Remdesivir, Aminotransferases, Hepatocytes, chicken

The effect of Remdesivir on the secretion of inflammatory markers by chicken liver cells

Zahra Akbari Jonoush[1], Roya Mahdavi[1], Maryam Farzaneh[2], Mehri Ghafourian[1]

1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Fertility, Infertility and Perinatology Research Center, Ahvaz JundishapurUniversity of Medical Sciences, Ahvaz, Iran

Background: For severe cases of SARS-CoV-2 infection (COVID-19), Remdesivir (RDV) is introduced as an anti-viral drug with side effects. Hepatotoxicity from prolonged exposure to RDV is associated with increased inflammatory factors. In this study, we evaluated the effect of RDV on the secretion of inflammatory markers by chicken liver cells.

Methods: 20 stage X embryonated chicken eggs were incubated for 10 days at 37.5°C and 60–65% humidity (stage HH35). In a medium containing DMEM/F12+10% FBS, liver cells were grown. After three days, the culture media was supplemented with four doses of RDV (1.00, 2.00, 3.00, and 4.00 M), after 24h and 48h, the viability of the hepatocytes, as well as gene expression of IL-1, IL-6, and TNF-αwere assessed.

Results: Each hepatocyte had a prominent nucleus and nucleolus with a hexagonal shape. The pink tint of the Periodic acid Schiff (PAS)-positive cells in the PAS staining verified the hepatocytes' glycogen content. Up to 50% of the cells lose viability after 48 hours in the presence of 3 and 4 M RDV ($p< 0.001$). The expression of IL-1, IL-6, and TNF- α were all significantly raised after treatment with RDV ($p<0.001$).

Conclusion: We concluded that RDV therapy altered the expression and function of hepatocyte inflammatory factors

Keywords: Hepatocytes, COVID-19, Remdesivir, inflammatory factors

The effect of treatment with quercetin and antiviral drugs on serum levels of IL-6, IL1-β and TNF-αand laboratory indicators in severe COVID-19 patients: A randomized controlled trial

Mojtaba Shohan[1], Ali Khodadadi[1]

1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Background: COVID-19, an infectious disease affecting the respiratory system, is occurred after being infected by SARS-CoV-2. The most available treatments include oxygen therapy, corticosteroids, and antiviral drugs. Although there are controversial reports regarding the therapeutic effectiveness of remdesivir and favipiravir, the latest studies reported their potential in shortening the time to clinical improvement in severe COVID-19 cases. This study aimed to assess the therapeutic effectiveness of quercetin, as a complementary treatment added to the antiviral drugs to reduce the serum levels of inflammatory cytokines and laboratory indicators related to severe COVID-19.

Methods: Through a randomized controlled trial, several 60 severe COVID-19 patients were allocated into 2 groups including control and intervention. During a 7-day period, patients in the control group received antivirals, i.e., remdesivir or favipiravir, while the intervention group was treated with 1000 milligrams of quercetin daily in addition to the antiviral drugs.

Results: According to the results, taking quercetin was significantly associated with reduced serum levels of ALP, q-CRP, and LDH in the intervention group. However, the serum levels of IL-1β TNF-α and IL-6 cytokines did not indicate any significant difference between the 2 groups.

Conclusion: Based on our observations, quercetin is safe and effective in lowering the serum levels of ALP, q-CRP, and LDH as critical markers involved in COVID-19 severity. However, according to the non-significant borderline results in comparing the serum levels of IL-1β TNF-α and IL-6 cytokines, further studies can be helpful to compensate for the limitations of our study and clarify the therapeutic potential of quercetin in COVID-19 treatments.

Keywords: COVID-19, Remdesivir, Favipiravir, Inflammatory Cytokines

The first report of 2:1 atrioventricular block following COVID-19 vaccination

Seyyed Shamsadin Athari[1], Entezar Mehrabi Nasab[2]

1. Zanjan University of Medical Sciences, Zanjan, Iran.
2. Tehran Heart Center, Tehran, Iran.

Background: The coronavirus infection disease 2019 (COVID-19), the recent pandemic ravaging the world, is a serious health threat to mankind. Current research on COVID-19 vaccines shows that most of their known side effects are mild and self-limiting and do not require any specific treatment.

Case presentation: In this case report, we presented a patient who developed a 2:1 AV block a few days after being vaccinated for COVID-19. A 65-year-old man, without a noticeable health condition or history of serious diseases, was referred to the emergency department of our hospital (Shohada Hospital).

Results: His main complaints were dizziness and mild dyspnea. The patient's symptoms started a few days after receiving a COVID-19 vaccine (Sinopharm). The initial 12-lead electrocardiography (12-lead ECG) showed sinus rhythms with 2:1 second-degree AV block (2:1 AV block), a narrow QRS complex, a ventricular rate of 37 bpm, and an atrial rate of 74 bpm. Chest CT scan showed no abnormality. Transthoracic echocardiography showed normal left ventricular size and left ventricular systolic and diastolic functions (LVEF = 55%). Also, the size and systolic function of the right ventricle were normal. There were no signs of valvular diseases, and pulmonary artery pressure (PAP) was normal (PAP = 15–20). A temporary pacemaker (TPM) was inserted, and the patient was closely observed for several days afterward, showing no AV block improvement. Therefore, a permanent pacemaker (PPM) was finally installed. After 1 week of admission to the hospital, the patient was discharged in good general condition, and he was recommended to visit for a follow-up.

Conclusion: In future studies, two issues should be addressed. First, the cardiac complications of COVID-19 vaccines should be more thoroughly evaluated to divulge the pathophysiology of the conduction disorders caused by these vaccines. Second, a suitable therapeutic method should be designed for patients developing AV block after COVID-19 vaccination.

Keywords: Atrioventricular block, COVID-19 vaccination, Pacemaker

The frequency of CD4+ HLA-G+ T lymphocytes, CD8+ HLA-G+ T lymphocytes and CD14+ HLA-G+ monocytes in patients with SARS-coronavirus-2

Mehdi Shahbazi[1], Somayeh Ramzannezhad[2], Mona Tarighi[1], Housein Ghorbani[2], Akramossadat Hosseini[2], Zahra Ahmadnia[2], Soudabeh Aghapour[1], Mousa Mohammadnia-Afrouzi[1]

1. Immunoregulation Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
2. Department of Pathology, School of Medicine, Babol University of Medical Sciences, Babol, Iran

Background: Coronavirus disease-2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Immune dysregulation leads to the inflammatory condition and massive production of inflammatory mediators that deteriorate patients' status. Here, regulatory immune cells may ameliorate the inflammatory conditions and improve the severity of the disease.

Methods: A total number of 76 participants were enrolled in this study and divided into three groups as follows; 25 moderate/severe and 26 critical COVID-19 patients as well as 25 healthy control subjects. After blood collection, peripheral blood mononuclear cells (PBMCs) were isolated and stained by FITC-conjugated anti-CD4 monoclonal antibodies (mABs), PE-conjugated anti-HLA-G mABs, PerCPCy5.5-conjugated anti-CD14 mABs, and APC-conjugated anti-CD8 mABs.

Results: The critical COVID-19 patients had a significantly lower frequency of CD4+ HLA-G+ T lymphocytes compared with moderate/severe patients (p-value < 0.001; SMD -1.27 95% CI [-1.86; -0.66]) and healthy control (p-value < 0.05; SMD -0.69 95% CI [-1.25; -0.12]). The critical COVID-19 patients had a significantly lower frequency of CD14+ HLA-G+ monocytes compared with moderate/severe patients (p< 0.001; SMD -2.09 95% CI [-2.77; -1.41]) and healthy control (p< 0.05; SMD -0.83 95% CI [-1.40; -0.25]). However, there was no difference between study subjects regarding the frequency of CD8+ HLA-G+ T lymphocytes.

Conclusion: Increased amount of immunomodulatory HLA-G+ cells may contribute to the low disease severity in moderate/severe compared with critical patients.

Keywords: COVID-19, SARS-CoV-2, HLA-G+ T cells, HLA-G+ monocytes

The prevalence of asymptomatic SARS-CoV-2 infection increases after vaccination

Mohammad Jafari[1], Ahmadreza Etemadi[1], Mina Bahoosh[2], Aliyar Pirouzi[1], Mehdi Mohsenzadeh[1]

1. Gerash University of Medical Sciences, Grash, Iran.
2. Estahban Division of Health Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Background: General vaccination against the covid-19 disease was widely carried out in Iran from September 1400. Vaccination reduces infection, serious illness, and death from SARS-CoV-2. However, breakthrough cases do occur, and this risk increases over time. Asymptomatic individuals play an important role in the SARS-CoV-2 transition. Therefore, this study aimed to assess the frequency of asymptomatic individuals after universal vaccination.

Methods: This retrospective study was conducted for 15 months from December 2020 to March 2022. Subjects tested were passengers on international flights referred to the Gerash Clinical and Molecular Diagnosis Laboratory. Passengers were divided into two groups. The former was those who came to our laboratory before September 1400, and the latter was those who came to our laboratory one month after the start of general vaccination. Diagnosis of SARS-Cov-2 infection was performed using Real-time PCR.

Results: Of the 9168 foreign passengers referred to our laboratory, 150 (1.63%) were positive for SARS-CoV-2 infection. In the unvaccinated group, 72 out of 5910 (1.22%) and in the vaccinated group, 78 of 3248 passengers (2.4%) had positive results for the SARS-CoV-2 test. The incidence of asymptomatic infections was significantly higher in the vaccinated group than in the unvaccinated group ($p< 0.001$).

Conclusion: Considering the role of asymptomatic individuals in the transmission and spread of Covid-19 disease, a focus on second-generation vaccines to prevent not only disease but also infection is recommended and should be a priority for the World Health Organization.

Keywords: SARS-CoV-2, asymptomatic individuals, Vaccination

The quantity and quality of anti-SARS-CoV-2 IgG antibodies are inversely correlated with COVID-19 severity: Lessons learned from IgG avidity

Mohsen Rastegari-Pouyani[1], Ghasem Solgi[1], Mehrdad Hajilooi[1]

1. Hamadan University of Medical Sciences, Hamadan, Iran.

Background: Gaining more appreciation of the protective or/and detrimental aspects of anti-SARS-CoV-2 immune responses associated with COVID-19 severity is of key importance. In the current study, we aimed to explore the avidity of serum IgG antibodies to SARS-CoV-2 spike (S) and nucleocapsid (N) antigens in severely ill symptomatic COVID-19 patients and asymptomatic RT-PCR confirmed SARS-CoV-2 carriers as well as to compare avidities of these antibodies about vaccination status, vaccination dose, and re-infection status.

Methods: Serum anti-S and anti-N IgG levels were measured by specific ELISA kits. Antibody avidity was evaluated by urea dissociation assay and expressed as avidity index (AI) values.

Results: Though symptomatic patients showed higher IgG levels, AI values of both anti-S and anti-N antibodies were significantly lower in this group in comparison to asymptomatic carriers. In both groups, the avidity of anti-S antibodies was higher in one-dose and two-dose vaccinees versus unvaccinated cases, although significant differences were only found in the symptomatic group. However, vaccinated and unvaccinated subgroups showed no significant difference in anti-N IgG avidity. Roughly all vaccinated patients of different subgroups (based on vaccine type) showed higher anti-S IgG avidity whereas the statistical significance was only detected between those receiving Sinopharm compared with the unvaccinated subgroup. Additionally, statistically significant differences in antibody AI values were detected only between primarily infected subjects of the two groups.

Conclusion: Our findings highlight a crucial role for the avidity of anti-SARS-CoV-2 IgG antibodies in protection from severe COVID-19, and calls for the incorporation of antibody avidity assessment into the currently used diagnostic tests to predict effective immunity against SARS-CoV-2 infection or even for prognostic purposes.

Keywords: COVID-19, Symptomatic, Asymptomatic, IgG avidity

Toxoplasmosis impact on hospitalized patients with moderate and severe Covid-19

Seyedeh Zohreh Jalali[1]

1. Department of Neonatology, School of Medicine, Guilan University of Medical Sciences Rasht, Guilan, Iran

Background: Coronavirus Disease 2019 (COVID-19) has raised a global health emergency. Treatment of COVID-19 remains a complicated challenge and immunosuppressive especially corticosteroids has shown promising therapeutic results. However, there is a risk of reactivation of opportunistic infections such as Toxoplasma gondii with immunosuppressive agents. This study was designed to increase information about toxoplasmosis in COVID-19 patients in Guilan province, Iran.

Methods: The study was performed among 85 patients with an RT-PCR-positive test for COVID-19, hospitalized from January 2022 to December 2022. A questionnaire, including demographic and epidemiological information, was completed. Peripheral blood samples were taken for each participant and separated serum was kept at −20 °C until use. IgG and IgM antibodies to T. gondii were detected by a commercial enzyme-linked immunosorbent assay (ELISA) kit. Variables related to the COVID-19 severity and outcomes were analyzed.

Results: All patients with COVID-19 through RT-PCR, 50 (58.8%), and 35 (41.2%) suffered from moderate and severe COVID-19, respectively. Anti-T. Gondii IgG was detected in 72.0 % and 82.9% of patients with moderate and severe COVID, which was not statistically significant (p=0.245). There were no significant differences between the demographic characteristics of participants in the two groups of moderate and severe COVID-19. In the comparison of moderate and severe COVID-19, mortality in people with positive anti-T gondii IgG was statistically significant (p=0.001).

Conclusion: Collectively, the results from the present study revealed a high percentage of positivity for Toxoplasma IgG antibodies in COVID patients. These patients are at high risk for re-activation of toxoplasmosis infection. We suggest that COVID patients should be regularly monitored to avoid the risk of re-activation of chronic toxoplasmosis

Keywords: COVID-19, Toxoplasmosis, Mortality

Environmental Pollution and Immunology

Evaluation of Diesel exhaust particles (DEPs) effects on inflammasome pathway in experimental model of asthma

Sheyda Mohammadkhani[1], Hamid Reza Nouri[2]

1. Babol University of Medical Sciences, Babol, Iran.
2. Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences.

Background: Diesel exhaust particles (DEPs) can exacerbate asthma due to bronchitis. Recent studies indicate the role of inflammatory processes, including increased production of inflammatory cytokines in asthma. The study of inflammatory pathways involved in the production of inflammatory cytokines, is in the primary steps. Therefore, we aimed to investigate the inflammasome pathway to produce IL-1βin the experimental model of asthma exposed to DEPs.

Methods: In this study, BALB/c female mice were divided into six groups: control, alum, asthmatic, and asthmatic treated with two doses of DEPs and DEPs. To induce asthma, the mice were first sensitized by three intraperitoneal injections of ovalbumin (20 µg) with alum adjuvant (2 mg/ml) and then subjected to pulmonary challenge with 1% albumin. To evaluate the effect of DEPs, mice were exposed to different concentrations of DEPs (2, 20 mg / m3). Serum, tissue, and bronchoalveolar lavage fluid were collected after the last dose of exposure.

Results: The results of H & E and PAS staining showed increased cell infiltration, thickening of the alveolar septum, increased smooth muscle thickness around bronchioles, and increased mucus secretion in the asthmatic group compared with the control group. These factors showed a significant increase due to treatment with DEPs. Expression of inflammasome-related genes by qPCR showed that following the induction of asthma, AIM2 and IL-1β expression increase significantly (p<0.05). In contrast, the expression level of NLRP3, NAIP, NLRC4, ASC, Caspase-1, and IL-18 showed no significant difference compared to the control group. NLRC4 gene expression in the asthmatic group exposed to low-dose DEPs showed a significant increase (p<0.05) compared to the control and asthma groups. Also, in asthmatic mice exposed to high doses of DEPs, a considerable decrease in the expression of AIM2 and NLRC4 genes was observed (p<0.05). DEPs alone also increased the expression level of the IL-18 gene compared to the control group. However, measurement of IL-1βprotein level by ELISA showed an increased level in this cytokine in lung tissue in the asthmatic group exposed to high doses of DEPs.

Conclusion: Based on the results, it can be concluded that the increase in IL-1βproduction due to exposure to low doses of DEPs is probably due to the activation of the NLRC4 inflammatory complex, which may play a major role in exacerbating asthma. On the other hand, decreased expression of AIM2 and NLRC4 genes in asthmatic mice exposed to high doses of DEPs reinforces the possibility that DEPs may be involved in exacerbating asthma from other pathways besides inflammation.

Keywords: Asthma, Cytokine, DEPs, Inflammasome.

Geo-climatic variability and adult asthma hospitalization in Fars, southwest Iran

Zahra Kanannejad[1], Mohammad Amin Ghatee[2], Soheila Alyasin[2]

1. Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Parasitology, School of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran.

Background: Asthma is a chronic respiratory disease resulting from a complex interaction between genetic and environmental factors. Many environmental factors have been associated with incidence or prevalence of asthma although there is still limited knowledge of major environmental causes of asthma in the general population. This study, for the first time, investigated the effects of climatic and geographical variability on asthma hospitalization among an adult population living in Fars province, southwest Iran.

Methods: During the study period, the home addresses of patients hospitalized with acute asthma from 2016 to 2019 were mapped. The effects of geo-climatic factors including temperature, rainfall, and humidity, and evaporation, number of rainy and frosty days, slope, and land covers were assessed on adult asthma hospitalization by Geographical Information System (GIS). Data were analyzed using univariate and multivariate binary logistic regression.

Results: A total of 349 patients were recruited, including 157 (44.98%) males and 192 (55%) females. The mean age was 57.77±18.84 years, ranging from 19 to 98 years. Asthmatic patients came from a total of 82 points including villages, towns, and cities. In the univariate analysis, urban setting (OR=13) and Mean Annual Rainy Days (MARD) (OR=1.03) were identified as the factors associated with increased asthma hospitalization, while Mean Annual Temperature (MAT) (OR=0.927), MinMAT (OR=0.933), MaxMAT (OR=0.925), Mean Annual Evaporation (MAE) (OR=0.999), and slope (OR=0.925) negatively affected asthma hospitalization. Urban setting was considered the only significant factor in multivariate analysis (OR=11.026).

Conclusion: The major risk zones for acute asthma in southwest Iran were urban settings and areas with higher numbers of rainy days, lower temperatures, and evaporation at lower slopes.

Keywords: asthma hospitalization, climatic changes, geographical factors, land cover, GIS

Sensetization antigens of brown-banded cockroach (Supella longipalpa) and compartion with German cockroach (Blattella germanica) in the animal model

Amin Asadollahi[1], Hamidreza Basseri[1], Ali khamesipour[2], Maryam Sharif Shoushtari[3], Mozhgan Baniardalani[1], Zahra pourpak[4], Ali Akbar Sabour yaraghi[5]

1. Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences Tehran, Iran
2. Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, Tehran, Iran
3. Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
4. Immunology, Asthma, and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Pathobiology, School of Public Health Food Microbiology Research Center, Tehran University of Medical Sciences

Background: Cockroaches are one of the most important causes of asthma and allergies in residential areas. Sensitization to cockroach allergens is an established risk factor for asthma among populations.

Methods: In this study, Cockroach hole bodies and feces of all two species were separately dried and homogenized, and centrifuged after one day of staying in PBS (phosphate buffered saline). The supernatant was prepared for injection into 20 mice and after four weeks of subcutaneous injection. The protein concentration of the samples was determined using the Bradford method. The ELISA test was used to determine the antibody titer against the injected antigen. After detection of OD (overdose), an SDS-PAGE test was performed to determine the molecular band and molecular weight of the peptides, and then immediately Western blot test with HRP (Horseradish Peroxidase) antibody IgG conjugated mouse for total IgG, and then HRP anti-IgE conjugated mouse for IgE in rat serum done.

Results: Comparison of the cockroach OD with the rest showed that more antibodies, both for feces and for the body, were produced in the mouse serum than in other cockroaches. Western blot test also showed that the body of this cockroach produced IgE antibodies in the serum of mice.

Conclusion: The upper titer of the whole body and the Cockroach feces of the Supella longipalpa were observed in comparison with the other cockroaches, and the IgE antibody band of the Supella hole body was observed, which indicates the risk of allergy to this cockroach.

Keywords: Supella longipalpa, Blattella germanica, SDS-PAGE, Western blot

The relationship between children's exposure to cigarette smoke and the prevalence of asthma and allergy symptoms

Afshin Mohebbi Zarin Dare[1], Zeinab Sadat Moosavifard[2]

1. Bachelor of Nursing student, Young and Elite Researchers Club, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran
2. Faculty, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran

Background: Asthma is one of the most chronic diseases in children and is of great importance due to its higher prevalence among children than adults. Genetic factors are not ineffective in causing asthma, but environmental factors have the greatest impact. Cigarette smoke is the worst cause of asthma for children, which may not be cured even with medicine. Considering that the immune system and lungs are developing at a young age, contact with these substances at this age can have harmful effects. It seems that exposure to cigarette smoke is not a cause of wheezing attacks, but rather an aggravating factor. This study was designed and implemented with the aim of identifying the relationship between environmental exposure to cigarette smoke and the onset and severity of asthma and allergy symptoms in Shiraz.

Methods: The study was carried out in a cross-sectional descriptive method among students. The sampling method in schools was a simple random cluster and in each school it was a census among students. A standard Lisak questionnaire was used to collect data. The questionnaires were analyzed in the SPSS software environment using chi-square and ANOVA tests.

Results: In this study, 1509 students aged 7-9 and 1001 teenagers aged 14-16 were examined. Fathers of 39.8% of the studied population were smokers. Prevalence of asthma symptoms in both age groups and allergic rhinitis in the age group of 14-16 in children where a smoker lived at home. It is more with a significant difference. Exposure to hookah smoke at home and the presence of a smoking mother in children aged 7-9 have significantly increased the symptoms of allergic rhinitis. In 14-16-year-olds, the presence of a smoking father caused a significant increase in allergic rhinitis, and the presence of a smoker at home caused a significant increase in the frequency of wheezing and allergic rhinitis symptoms.

Conclusion: The results of this study show that exposure to cigarette smoke during childhood increases the risk of atopic diseases, especially asthma, rhinorrhea and conjunctivitis. Exposure to tobacco smoke during childhood can increase the likelihood of asthma in adulthood. Attention of parents regarding the harm of children's exposure to cigarette and hookah smoke in the occurrence or exacerbation of asthma and allergy symptoms in them and the need to avoid this issue. It is recommended.

Keywords: Children, exposure, cigarette smoke, asthma, allergy

Ethics in Immunological Studies

Obtaining informed consent in immunology and immunotherapy and related laws in Iran

Alireza Ghassemi Toussi[1]

1. Department of Clinical Toxicology, School Of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: In Iran Setting up a consent form to perform medical procedures such as immunology and immunotherapy is not a new thing and has been done for a long time. Consent of a person under 18 years of age being get from the patient's guardian and over 18 years of age from the alert patient herself or himself.

Methods: Today, in Iran due to the more complicated process of immunology and immunotherapy, informing the patient about the probability of success of the treatment, possible risks, side effects of the treatment, is considered an inseparable part of the treatment measures, and such information can be considered as a major obstacle in obtaining informed consent.

Results: Informed consent is one of the most fundamental concepts in medical ethics and patient rights in the world especially in Iran, in such a way that its conscious regulation before the start of any diagnostic and therapeutic activity in immunology and immunotherapy will lead to positive moral and clinical results.

Conclusion: Medical staff are obliged to provide patients with information about the immunology and immunotherapy and related procedure and diagnostic test and treatment, and a doctor who treats the patient without the patient's consent will be prosecuted

Keywords: consent, immunology, immunotherapy, Iran

Legal aspects in cancer immunotherapy

Alireza Ghassemi Toussi[1]

1. Department of Clinical Toxicology, School Of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: nowadays, complaints against the medical staff due to Negligence about the treatment of cancer with immunotherapy is increasing. Negligence in the legal, means that does not perform assigned task. The task that the legislator has placed on that person. The legislator in the Islamic Penal Code, adopted in latest version, defines negligence as carelessness or omission and also includes lack of skill and non-compliance with state regulations as part of this.

Methods: The definition of the above items is in brief: Omission: Non performance of an act which scientifically and technically is expected to be done. Carelessness: Performance of an action which scientifically and technically should not be done. Lack of skill: Includes cases in which the physician does not have the either scientific or technical skill for a certain work. Failure to comply with government regulations: Namely, failure to pay attention to regulations, departmental letters, regulations of administrative superiors, medical system, Ministry of Health.

Results: Examples of the four listed in Materials and Methods above, includes the following1-in a elderly patient with pain in pelvic and history of falling not request x ray before starting immonutherapy.2- Prescribing gentamicin for a 80 years old patient without adjusting dose in cancer immunotherapy and rising of creatinin.3- Incorrect Injection of immunotherapic agent in patient.4-: Not obtaining informed consent in necessary cases in a cancer patient and start immunotherapy.

Conclusion: Due to the special conditions of patient who need to starting immunotherapy, doctors should be more careful in examining and taking history from themselves and their relatives so as not to make mistakes in the diagnosis and treatment of them.

Keywords: immunotherapy, omission; carelessness; Lack of skill; government regulations

Exercise Immunology

Anti-inflammatory Effects of 6 Weeks of Interval Swimming Training on PGC-1α Gene Expression in the Biceps Muscle Tissue of Male Wistar Rats

Elham Shahabpour[1], Zahra Mirakhori[2]

1. Assistant Professor of Exercise Physiology, Department of Sport Sciences, Faculty of Humanities Sciences,University of Hormozgan, Bandar Abbas,Iran.
2. Assistant Professor, Department of Physical Education, Amirkabir University of Technology,Tehran,Iran

Background: Peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) acts as a potential mediator of exercise-induced adaptations in skeletal muscle that can regulate inflammatory mediators. Therefore, the present study was conducted with the aim of investigating the effect of 6 weeks of interval swimming training on PGC-1α gene expression in the biceps muscle tissue of male Wistar rats.

Methods: Male Wistar rats (N=20) were divided into 2 groups including interval swimming training (n=5), control (n=5). Mice swam for two weeks in order to adapt to the water temperature (25°C), then for 6 weeks, 3 days a week, the interval training protocol of swimming in the form of 2–3 minute intervals of swimming and 1 minute resting to maximum capacity was done between 3 and 10 sets with a training load of 3% to 6% of the body weight of the rats. The indicator of helplessness was the rat's inability to swim and go under water, as well as stick to the wall. 48 hours after the last training session and tissue samples were taken and stored at -70 °C. Biceps muscle tissue was homogenized in two research groups and the expression level of PGC-1α gene was measured by Real time-PCR method.

Results: The results showed that the level of PGC-1α gene expression in the biceps muscle of the interval swimming training group increased significantly compared to the control group (p=0.019).

Conclusion: These data support the beneficial effect of exercise training on PGC-1α acting as an essential factor for connecting metabolic regulation, redox control and inflammatory pathways, which may be partially mediated by the anti-inflammatory potential of exercise, which could be a target for helping it is an interesting treatment for some metabolic diseases.

Keywords: Swimming Interval Training, PGC-1α, Inflammation

High Intensity Interval Training and Intake nano-selenium supplementation on the gene expression of hepatic SOD in Dexamethasone Induced Rats

Motahare ghane[1], Shahin Riyahi Malayeri[1], Masume Hoseini[1]

1. East Tehran branch, Islamic Azad University, Tehran, Iran.

Background: Dexamethasone is one of the common glucocorticoids prescribed for humans and animals, which leads to oxidative damage by producing reactive oxygen species (ROS). Therefore, antioxidants can reduce tissue toxicity to prevent an increase in oxidative stress caused by dexamethasone. Objective The present study examines the combined effect of high intensity interval training (HIIT) and nano selenium supplementation on the expression of the hepatic superoxide dismutase (SOD) gene in dexamethasone-induced rats.

Methods: The samples were 40 Wistar rats in the age range of 8 weeks, which were randomly divided into 5 groups of 8 (healthy, induced by dexamethasone, dexamethasone + HIIT, dexamethasone + nano selenium supplementation, dexamethasone + HIIT + nano selenium supplementation) were divided. The exercise program consisted of 5-12 repetitions of 1-minute running with active rest intervals of 75 seconds. This training was performed six days a week for four weeks. The prepared stock solution of selenium nanoparticles was given to mice in the amount of 100 mg by gavage every other day.

Results: A two-way analysis of covariance was used to determine the difference in variables between groups at a significant level *($p<0.05$)*.The results showed that there was a significant increase in the expression of the hepatic SOD gene in the HIIT group with nano selenium compared to the control group *($p=0.034$)*.

Conclusion: Therefore, it seems that the intervention of high intensity interval training with the use of nano selenium supplements will lead to the strengthening of the antioxidant system and the improvement of the immune system, and reduce the adverse effects caused by the use of steroid drugs such as dexamethasone.

Keywords: Keywords High intensity interval training, Nano-selenium supplement, Dexamethasone, Superoxide dismutase

Home-based exercise training can modulate Transforming growth factor beta levels in Pregnancy

Mona Pourmohammad[1], Mahdieh Molanouri Shamsi[2], Lida Moghaddam-Banaem[1], Marzieh Moslemi Naeini[2]

1. Department of Reproductive Health and Midwifery, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Physical Education and Sport Sciences, Faculty of Humanities, Tarbiat Modares University, Tehran, Iran

Background: Overweight pregnant women are at higher risk of poor outcomes due to persistent inflammation. In addition, increased levels of TGF-β were found during high-risk pregnancies. In contrast, exercise training can regulate the changes in inflammatory factors. This study aimed to investigate whether exercise training affects the level of TGF-β factor in overweight pregnant women.

Methods: This study was performed as an experimental interventional study (a randomized clinical trial: RCT), to investigate the effect of combined exercise training on Transforming Growth Factor Beta (TGF-β) in overweight pregnant women. 25 pregnant women meeting inclusion criteria (BMI 25-29.9 kg/m2, single pregnancy, 16-18 weeks, without contraindications to exercise) were selected. Participants were randomly divided into two groups exercise intervention (n=13) and control (n=12) groups. In the intervention group, exercise consisted of aerobic, resistance, and stretching exercises five days a week from 16-18 until 36-37 weeks of pregnancy. The participants in both groups were assessed regarding blood pressure, weight, and anthropometric characteristics as well as maternal venous blood samples at 16-18, 25-29, and 36-40 weeks gestation. The control group continued their normal daily activities until the end of pregnancy. Finally, data analysis was performed with SPSS software v. 21.

Results: According to the results obtained based on linear regression analysis considering the effect of various factors along with exercise (Interleukin-10 and Hemoglobin), TGF-β levels were significantly lower in evaluations at 25-29 weeks of gestation in the intervention group than in the control group (460±325 vs. 620±662.5 pg/mL, p=0.019, β: -0.540). TGF-β was significantly lower in the intervention group than in the control group at 36-40 weeks of pregnancy (220±300 vs. 550±375 pg/mL, p=0.014).

Conclusion: Lower levels of cytokine TGF-β following exercise training during pregnancy may indicate a healthy pregnancy. More studies are needed to assess this novel finding.

Keywords: Combined Exercise training, Pregnancy, TGF-β, Overweight

Investigating the effect of anaerobic exercise time on the level of immunoglobulin M in young volleyball players

Arash Fathi[1], Zeinab Sadat Moosavifard[2], Mahsa Shekari[1]

1. Bachelor of Nursing student, Young and Elite Researchers Club, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran
2. Faculty, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran

Background: Immunoglobulins are the most important antibodies in human saliva that play an essential role in mucosal immunity and prevention of upper respiratory tract infections. Researchers emphasize the role of the humoral immune system in creating immunity against diseases. Immunoglobulin M is also of special importance as the first antibody that is synthesized and secreted in the immune response to infection. The purpose of this research was to investigate the effect of anaerobic training time on the immunoglobulin M of volleyball teenagers.

Methods: The following semi-experimental study was conducted on 40 teenagers in the age group of 13-18 years in the field of volleyball in Bandar Abbas city. In this research, two groups participated in the morning and evening. The morning training group (20 people) did their training at 9 in the morning and the evening training group (20 people) did their training at 6 in the evening. The training of both groups was similar 4 sessions a week and lasted for 6 weeks. The total time of the training program was about 60 minutes. After completing 6 weeks of training, the subjects went to Imam Hijab Bandar Abbas Hall to take the post-test at the same time as the pre-test. The technique used to measure salivary immunoglobulin M concentration was the nephelometric method. All statistical operations were performed using Spss statistical software.

Results: For the concentration of immunoglobulin M, there was no significant difference between the morning and evening sessions in any of the measurement steps. The mean immunoglobulin M of the evening shift group was higher than the morning shift group in all stages, and the morning shift group increased during the stages. Also, in the evening shift group, the average after the first week was higher than before the training, but after the 6th week it was lower than the first week.

Conclusion: According to the results of this research, it is better to exercise in the evening instead of exercising in the morning to increase the level of immunity.

Keywords: Salivary immunoglobulin, immunoglobulin M, anaerobic exercise, circadian rhythm, adolescent

Investigating the effect of exercise on changes in immunoglobulin A in female athletes and non-athletes at Shiraz University of Medical Sciences

Mahsa Shekari[1], Zeinab Sadat Moosavifard[2], Arash Fathi[1]

1. Bachelor of Nursing student, Young and Elite Researchers Club, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran
2. Faculty, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran

Background: Immunoglobulins play a key role in the body's immune system. They begin the process of getting rid of invaders that may cause damage or infection. Immunoglobulin A is in body secretions, saliva, colostrum and milk, which has the highest production compared to other antibodies, and is considered an important source of intestinal protection in the first few weeks of birth. The purpose of this research was to investigate the effect of 60 minutes of physical education on changes in salivary IgA of athletic and non-athletic female students between the ages of 20-24.

Methods: The current research was conducted using a semi-experimental method, the statistical population of which was female athletes and non-athletes between the ages of 20-24 at Shiraz University of Medical Sciences, who were studying in the academic year 1400-1401. The statistical samples of the research included 20 people who were randomly selected. 10 female athlete students with an average age of 21.45 ± 0.48 years, height 170 ± 30.6 cm, weight 83.53 ± 78.4 kg, and fat percentage 87.27 ± 77.2 and 10 non-athletic female students with an average age of 0.78 20.35 ± years, height 160 ± 20.6 cm, weight 76.53 ± 67.4 kg and fat percentage 78.27 ± 85.2. Both groups exercised for 60 minutes and the saliva samples of both groups were collected separately in three stages (before, immediately and 2 hours after the activity) and transferred to the laboratory, and the concentration of salivary IgA was measured by ELISA method was taken.

Results: The results showed that there is no difference between the salivary immunoglobulin A of female athletes and non-athletes before, immediately and two hours after performing 60 minutes of physical education, but in the analysis of variance in the repeated measurements of the two groups separately in three stages showed that in the athlete group; There is a significant difference between immunoglobulin A saliva before and immediately after the activity. In non-athletic students, there is a significant difference between salivary immunoglobulin A before and immediately after the activity and two hours later.

Conclusion: Exercise increases interferon and antibody production. A sport that is done continuously, regularly and with moderate intensity can certainly strengthen the immune system. Because performing regular sports activities causes the proliferation of lymphocytes. As a conclusion, it can be recommended that non-athletes avoid intense activities in physical training.

Keywords: Exercise, immunoglobulin A, female, athletes, non-athletes

Investigating the effect of exercise training along with the consumption of Lactobacillus rhamnosus bacteria on the expression of inflammatory chemokine CXCL2 and NF-κB in rats with steatosis

Hossein Shirvani[1], Fatemeh Rostamkhani[2]

1. Exercise Physiology Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Department of Biology, Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University, Tehran, Iran.

Background: It is known that exercise with optimal intensity and duration can strengthen the immune system. On the other hand, probiotics, while stimulating and strengthening the immune system of the digestive system, stimulate the immunity of other organs as well. The current study investigated the effects of HIIT and probiotic consumption on the expression of nuclear factor kappa B (NF-κB) and chemokine ligand 2 (CXCL2) genes in liver tissue in rats with steatosis.

Methods: For this purpose, 40 male Wistar rats with a weight range of 200-250 grams were randomly divided into five equal groups as follows: 1. healthy control group 2. Steatosis group 3. Steatosis+HIIT 4. Steatosis +probiotic 5. Steatosis + HIIT + probiotics. In order to induce steatosis, oral tetracycline 140 mg/kg/day in 2 ml of water in form of a solution was given to the rats by gavage for 7 days. After confirming steatosis in rats, Lactobacillus rhamnosus bacterium was also gavage at the rate of 109 CFU/day×5. HIIT exercise program performed on treadmill five sessions per week for 5 weeks. Forty-eight hours after the last training session, liver tissue sampling was done. The mentioned genes were measured by Real Time PCR method. One-way analysis of variance with Tukey's post hoc test was used for statistical analysis of the data at a significant level of $p<0.05$. All statistical studies were done using SPSS version 22 software.

Results: The results showed that the expression changes of NF-κB and CXCL2 liver genes in the steatosis group were significantly higher than other groups *($p=0.001$)* and was significantly lower in the HIIT and probiotic groups *($p=0.001$)*.

Conclusion: In general, it seems that the intervention of high intensity interval training with probiotic consumption can reduce the inflammation in the liver tissue by modulating the expression of NF-κB and CXCL2 genes and be used as an effective non-pharmacological method in the treatment of mild grades of fatty liver disease.

Keywords: High Intensity Interval Training, NF-κB, CXCL2, Steatosis, Lactobacillus rhamnosus Probiotic.

Investigating the effect of yoga breathing exercises on the control of asthma in adolescents with asthma referring to Shiraz Asthma and Allergy Clinic

Afshin Mohebbi zarin dare[1], Zeinab Sadat Moosavi fard[2]

1. Bachelor of Nursing student, Young and Elite Researchers Club, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran.
2. Faculty, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran.

Background: Asthma is a chronic disease of the respiratory tract that makes it difficult for a person to breathe and has affected about 300 million people in the world. Its symptoms usually include wheezing, coughing, chest tightness and shortness of breath. Background and purpose the goal of asthma treatment and management is to control it. One of the complementary treatments to control asthma is yoga. Yoga, whose popularity has spread globally, has the potential to alleviate some of the problems associated with asthma. This study was conducted with the aim of determining the effect of yoga breathing exercises on the control of asthma in adolescents with asthma.

Methods: Research method in this semi-experimental study, 50 adolescents aged 14-16 with asthma who referred to the Asthma and Allergy Clinic of Shiraz city in 2014 were included in the study through continuous sampling and non-random allocation in two experimental and control groups (25 people in each group). The data collection tool was the C-ACT asthma control questionnaire, which was completed before and after the intervention. The intervention included the implementation of yoga breathing exercises, which the researcher taught to the teenagers in the clinic, and which were performed by the teenagers at home for 3 months. Data were analyzed through SPSS software version 21 and using chi-square, Fisher, independent t and paired t tests.

Results: Findings The average age of the test group was 15.4 and the control group was 14.92. In the intervention group, 65.4% were boys, 34.6% were girls, and in the control group, 70.4% were boys and 29.6% were girls. The average score of asthma control before the intervention was 18.32 in the intervention group and 18.89 in the control group, and there was no statistically significant difference between the two groups ($p=0.54$). After the intervention, the average score of asthma control in the test group was 33.21 ± 1.6 and in the control group. 17.98±2.4 was reported and a statistically significant difference was observed between the two groups ($p<0.001$).

Conclusion: Practicing yoga breathing exercises helps with breathing and stress management, both of which are widely known to improve asthma. According to the results of the research, exercises such as deep breathing and relaxation as well as movements that help to open the chest are recommended as scientific, simple and uplifting techniques for these patients.

Keywords: yoga, breathing exercises, asthma, adolescents

Irisin have anti-inflammatory effects in response to exercise

Elham Shahbpour[1], Zahra Mirakhori[2]

1. Department of Sport Sciences, Faculty of Humanities Sciences, University of Hormozgan, Hormozgan, Iran.
2. Department of Physical Education, University of Amir Kabir, Tehran, Iran.

Background: Irisin has anti-inflammatory effects on adipocytes and macrophages. As it improves their ability for phagocytosis and reduces the intensity of processes related to ROS production. So the aim of the present study were to investigate the effect of swimming training on muscle irisin expression.

Methods: 25 male Wistar rat (age 8 weeks and weight 250 ± 30gr) after being familiarized with the laboratory environment were randomly divided into swimming and control group. Rats swam 3 days a week for 6 weeks. 48 hours after the last training session, rats were sacrificed and their blood samples was taken. Then the ELISA method was used to measure the irisin gene. One-way ANOVA test was used to analyze the findings. Finally T-test was used to analyze the findings.

Results: muscle irisin levels were significantly increased in training group compared to the control group ($p<001$).

Conclusion: 6 weeks swimming increased Irisin plasma levels in rat. It seems that Irisin exerts its positive anti-inflammatory effects in RAW 264.7 macrophages through down-regulation of TLR4/MyD88 downstream pathways.

Keywords: anti-inflammatory, irisin, aerobic training

Regular Physical Exercise can change Interleukin-10 and Tumor Necrosis Factor Alpha in Pregnant women

Mona Pourmohammad[1], Lida Moghaddam-Banaem[1], Mahdieh Molanouri Shamsi[2]

1. Department of Reproductive Health and Midwifery, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Physical Education and Sport Sciences, Faculty of Humanities, Tarbiat Modares University, Tehran, Iran

Background: As a result of excess adipose tissue and chronic inflammation, overweight pregnant women's inflammatory systems have more complications and may lead to poor outcomes. In contrast, exercise can modulate inflammatory factors. Therefore, this study examined the effects of Home-based Combined Exercise training on inflammatory responses in overweight pregnant women.

Methods: This study was performed as an experimental interventional study (a randomized clinical trial: RCT), on 25 pregnant women who met the inclusion criteria (BMI 25-29.9 kg/m2, single pregnancy, 16-18 weeks, without contraindications to exercise). Participants were randomly divided into two groups: exercise intervention (n=13) and control (n=12). A five-day-a-week exercise program consisted of aerobic, resistance, and stretching exercises performed between 16-18 weeks and 36-37 weeks. During 16-18, 25-29 and 36-40 weeks of gestation, participants were evaluated in person for blood pressure, weight, and anthropometric characteristics, and maternal venous blood samples were taken. The control group continued prenatal care and normal daily activities until delivery. Finally, data analysis was performed with SPSS software v. 21.

Results: According to the results of linear regression analysis, TNF-α levels in both evaluations at 25-29 and 36-40 of gestation, were significantly lower in the intervention group than the control group (23.97±2191 vs. 74.01±33.63, $p=0.034$, β: -0.415 and 22.5±16.44 vs. 83.79±37.05 pg/ml, $p= 0.015$, β: - 0.471, respectively). Also, based on linear regression analysis the (TNF-α/IL-10) ratio at (36-37) gestation was significantly lower in the intervention group (1.07±0.51 vs. 1.29±1.97, $p=0.022$, β: -.411).

Conclusion: The results of the present study showed that performing Home-based combined exercise training in overweight pregnant women can be effective in controlling TNF-α pro-inflammatory factor and (TNF-α/IL-10) ratio at the end of the third trimester. Since this study was the first of its kind about pregnancy, combined exercises, and inflammatory responses, further studies with greater sample sizes are needed.

Keywords: Home-based Combined Exercise, Pregnancy, TNF-α, Overweight

The effect of 8 weeks of aerobic exercise on the serum levels of CRP and IL-17 in Covid-19 patients after the treatment of the disease

Arman Jalili[1], Hossein Ali Khazaei[2], Ali Ali Dadi[3], Zainab Ali Dadi[4]

1. Sports Physiologist (PHD) - Manager of the Sports Complex, Hes Khob Fitness, Zahedan, Iran
2. Professor of Immunology, Clinical Immunology Research Center at Zahedan University of Medical Sciences, Zahedan-Iran
3. Associate Professor of Nephrology Clinical Immunology Research Center at Zahedan University of Medical Sciences, Zahedan-Iran
4. Director of the Sports Complex, Hes Khob Fitness, Zahedan, Iran

Background: In December 2019, a disease called the Coronavirus was detected in the city of Wuhan, China, which spread rapidly in the world. This deadly virus was named Covid-19 by the World Health Organization. Reports indicate that on April 15, Iran identified 76,389 people as patients with the coronavirus, of which 4,777 people died due to this disease. The Covid-19 virus is a disease that causes respiratory disease in people and can spread from person to person. An increase in inflammatory markers levels is one of the characteristics of this viral disease. Due to the novelty of the coronavirus, there has not been coherent scientific research on the corona disease, especially in the field of the effect of sports activity and training on its various dimensions. However, based on the physiological characteristics of corona disease and the effect this disease has on the body's immune system, the impact of exercise on inflammatory levels was investigated in this article.

Methods: This study was a semi-experimental type, in which there were 60 patients with covid-19 disease, that have passed two weeks since the end of their treatment process and the PCR covid test had also become negative, without respiratory disorders, with an average age of 35 ± 5 were invited to cooperate in a study. In this research, the patients were divided into 2 groups, case, and control. The patients of the case group experienced 8 weeks of aerobic training with 65-70% of the previous maximum heart rate. The patients in the control groups did not have any exercise or medication. Blood samples were collected the day before and one day after training. The levels of CRP and interleukin 17 were measured by the ELISA method.

Results: In the control group, which did not have any exercise or medication, no significant difference was observed in the serum level of CRP and IL-17 whereas, in the case of the group, the serum levels of CRP and IL-17 decreased significantly before and after exercise ($p<0.01$).

Conclusion: Aerobic exercises have a significant effect on reducing inflammatory indices in patients with covid-19.

Keywords: Covid 19 disease, aerobic exercise, CRP, IL-17

Immunodeficiency

A rare immunological disease, Caspase 8 deficiency

Hedieh Haji Khodaverdi Khani[1], Zahra Chavoshzadeh[2], Samin Sharafian[3]

1. Department of Immunology, School of Medical Science, University of Shahed, Tehran, Iran.
2. Department of allergy and clinical Immunology, Mofid's Children Hospital, University of Shahid Beheshti, Tehran, Iran.
3. Department of Immunology and Allergy, Mofid's Children Hospital, University of Shahid Beheshti, Tehran, Iran.

Background: Caspase 8 is a molecule in the FAS pathway that causes apoptosis. Caspase 8 deficiency is a rare autoimmune lymphoproliferative syndrome characterized by immunodeficiency, splenomegaly, and lymphadenopathy.

Case Presentation: In this study, we reported a two-year-old boy who had a fever of unclear cause and dysentery. He also had failure to thrive and was allergic to cow's milk protein. Antibiotics had no effect on his fever and dysentery. Due to dysentery, he had colonoscopy, which revealed diffused ulceration regions in the sigmoid with skipped areas, mimicking Crohn's disease aphthous lesions. Infantile inflammatory bowel disease (IBD) was diagnosed as a result of Caspase 8 deficiency.

Conclusion: Diarrhea or dysentery may be the first or predominant presentation of inborn errors of immunity (IEIs). The cause of the diarrhea and dysentery in this case was Infantile IBD. Infantile IBD is a symptom of IEIs, one of which is Caspase 8 deficiency. T cell counts in infants were normal. Although immunoglobulin levels may be normal or low, the immunological response was inadequate.

Keywords: Inborn errors of immunity, Infantile IBD, Caspase 8 deficiency, Immunodeficiency

Antibody Production after COVID-19 vaccination in Primary Immunodeficiency Patients

Maryam Nourizadeh[1], Elham Feizabadi[1], Milad Mirmoghtadaei[1], Ashraf Mohammadi[2], Mohammadreza Fazlollahi[1], Zahra Pourpak[1]

1. Immunology, Asthma and Allergy Research Institute (IAARI), Tehran University of Medical Sciences (TUMS), Tehran, Iran
2. Human Viral Vaccines Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

Background: Vaccinating patients with primary immunodeficiency (PID) against COVID-19 is a rational approach to counteract the disease in these patients. Few studies have evaluated COVID-19 vaccine efficacy in PID patients. This study compared antibody production after COVID-19 vaccination in 42 PID patients and 32 controls.

Methods: Three methods were used to measure antibody production, including the SARS-CoV-2 neutralization test, anti-SARS-Cov-2 immunoglobulin titer, and anti-SARS-Cov-2 neutralizing antibody test (ChemoBind).

Results: The patient's ages ranges from 19 to 78, with a median of 33, the median age for the controls was 41 years. PID cases included hereditary angioedema (n=19), common variable immunodeficiency (n=8), CGD (n=7), neutropenia (n=3), hyper-IgE syndrome (n=2) and X-linked agammaglobulinemia. Of the 42 patients, 40 (93%) had received Sinopharm. The findings of this study suggest that PID patients in most subgroups had antibody production similar to that of controls. Lower hospitalization rates were also observed in these patients.

Conclusion: These effects could be explained by the restrictive measures taken by PID patients, the non-significant role of B cells and antibody protection against COVID-19, or the lower likelihood of immune system overactivation. More evidence is needed to establish effective guidelines on the type and schedule of vaccines in different PID subgroups.

Keywords: PID, COVID-19, Neutralizing antibody

Comparison of Clinical Manifestations, Immunological Analyses between LRBA and CVID Patients: A Longitudinal Study

Maryam Sharifi[1], Javad Tafaroji[2], Alireza Sharifi[3]

1. MD Student, Student Research Committee, Iran University of Medical Sciences, Tehran, Iran
2. Department of Pediatrics, Qom University of Medical Sciences, Qom, Iran
3. MD Student, Student Research Committee, Qom University of Medical Sciences, Qom, Iran.

Background: Common variable immunodeficiency (CVID), is generally recognized as the most frequent type of Symptomatic primary immunodeficiencies (PID). Mutations in lipopolysaccharideresponsive beige-like anchor protein (LRBA) gene, are the most common genetic alterations amongst CVID patients. To date, there are no published studies to compare clinical and immunologic features of LRBA-deficient patients with those who do not harbor any known genetic mutations. Therefore, this study aims to compare the clinical manifestations and laboratory findings of Iranian patients with LRBA-deficiency and CVID with no known genetic alterations.

Methods: We performed a longitudinal study on patients who had been diagnosed with CVID. Demographic and clinical features were obtained via the databank of the Iranian Registry of Primary Immunodeficiencies, and the direct interviews with patients. To assess the presence of LRBA or other genetic mutations, whole-exome sequencing (WES) was used. Immunologic characteristics of patients were evaluated using flow cytometry, nephelometry, and conventional blood counts. The current study is conducted at Tehran's Children Medical Center and is approved by the ethics committee of Tehran University of Medical Sciences.

Results: Between March 2013 and October 2019, we enrolled 30 patients with LRBA-deficiency and 13 patients with CVID, who had no identified genetic mutations. Regarding clinical features, there were no significant differences for the prevalence of infections at different sites (lung, sinuses, and middle ear) among the two groups (all $p>0.05$). However, the incidences of autoimmune disorders and enteropathy were significantly higher among LRBA-deficient cases ($p<0.001$). In serum levels of immunoglobulins, there were significant differences for IgG and IgM between the two groups (*p of 0.014 and 0.004*, respectively); however, this was not seen for IgA and IgE levels. Likewise, we did not see any significant differences for the cluster of differentiation (CD) markers between the two groups (all $p> 0.05$).

Conclusion: Compared to the CVID patients with no identified genetic mutations, LRBA-deficient patients have a significantly greater chance of parental consanguinity and developing autoimmune disorders and enteropathy, and have significantly higher values of serum IgG and IgM. The rate of infectious complications and other basic laboratory features, do not show significant differences between the two groups.

Keywords: Common Variable Immunodeficiency, Lipopolysaccharide-responsive, Beige-Like Anchor Protein, Immunodeficiency, Autoimmunity, IBD-Like Syndrome, Enteropathy

Comprehensive Assessment of Skin Disorders in Patients with Common Variable Immunodeficiency (CVID)

Ali Zarezadeh Mehrabadi[1], Nazanin Aghamohamadi[1], Hassan Abolhassani[2], Asghar Aghamohammadi[3], Nima Rezaei[3], Reza Yazdani[4]

1. Immunology Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Division of Clinical Immunology, Department of Bioscience and Nutrition, Karolinska Institute at Karolinska University Hospital Huddinge, Stockholm, Sweden
3. Research Center for Immunodeficiencies, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Neurology, Thomas Jefferson University, Philadelphia, PA, 19107, USA

Background: Common variable immunodeficiency (CVID) is an inborn error of immunity (IEI) characterized by various clinical manifestations such as hypogammaglobulinemia, recurrent infections, and autoimmune diseases. Among different clinical manifestations, skin manifestations have been less reported in these patients.

Methods: In this study, we investigated the prevalence of dermatologic features in 387 CVID patients. Demographic information, clinical manifestations, laboratory data, and genetic findings were collected from medical records. All data were analyzed based on the presence or absence of skin disorders in CVID patients.

Results: We observed at least one skin manifestation in about 40% of these patients. Among these complications, skin infection (n = 64, 42.1%) was the most frequent presentation, followed by non-infectious skin lesions (n = 54, 35.6%). Among skin infections, abscesses (n = 34, 22.4%) were the most common complication. Skin infections such as cellulitis, impetigo, measles, and warts were also documented. Eczema (n = 34, 22.4%) was the most common complication in atopic lesions, and vitiligo (n = 13, 8.5%) was prevalent in autoimmune/pigmentation disorders. Among all the patients with genetic mutations, one-quarter had a deleterious mutation in the LRBA gene, relating to the autoimmune and atopic skin lesions.

Conclusion: This rate of skin disorders in our cohort demonstrating these manifestations could be significant in CVID patients, and they are not rare. Low data of skin complications in CVID patients could be attributed to insufficient attention of physicians and also might alert dermatologists to perform immunological investigations in children with certain skin manifestations.

Keywords: Common Variable Immunodeficiency, Skin Disorders

Different manifestations of chronic granulomatosis disease in a family with the same gene mutation

Shahrzad Fallah[1], Zahra Chavoshzadeh[1]

1. Department of Allergy and Clinical Immunology, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Chronic granulomatosis disease (CGD) is a severe, rare inherited form of inborn errors of immunity. Patients suffer from this disease experience recurrent bacterial, fungal infections, auto inflammatory process and sometimes granuloma formation. CGD is inherited in either X-linked or autosomal recessive manner. Different gene mutation can lead to this disease. CGD often manifest in childhood but sometimes disease presentation can be later. Here, we present two cases of CGD (Father and son) with different presentations despite the same type of genetic defect. This can highlight importance of gene penetrance and difference in expressivity in primary immune deficiency diseases.

Case presentation: A 26-month-old boy from close relative parents admitted in Mofid children hospital because of prolonged fever and left submandibular lymphadenitis. He treated with broad spectrum antibiotic. Lymph node excisional biopsy was performed and lymph node secretion culture showed presence of serratia marcescens. Immunological work up performed and the nitro blue tetrazolium (NBT) test value was zero. He was diagnosed with CGD and whole exome sequencing performed because of prenatal diagnosis. Test showed homozygous deletion mutation in NCF1 gene (NM-000265.6:exon2:c.75-76del). Sanger sequencing was done and same homozygous mutation was reported in his father. His father had no remarkable history except period of aphtus lesion and tooth decays.

Conclusion: This study shows us significance of gene penetrance in disease manifestations. We must be aware that Variable genetic penetration can challenge the expectations of physicians from the manifestations of genetic diseases.

Keywords: Chronic granulomatosis disease, gene penetrance

Examination of Genetic cause of an immune deficiency patient with hyper IgE

Samira Bahrami[1]

1. Immunodeficiency Diseases Research Center, Alzahra Hospital University, Esfahan University of Medical Science, Esfahan, Iran

Background: T0 date inborn errors of immunity (IEI) include more than 400 various diseases caused by >400 various responsible genes. The overall incidence of IEIs is ~1:10,000. However, this incidence is higher in some countries like Iran because of high consanguinity and fertility rates. Next-generation sequencing as a high- throughput technique (like whole exome sequencing (WES),) has improved the genetic diagnosis for IEI patients. In this study, we aimed to detect the genetic cause of disease in a 35 year old woman patient with autoimmunity (SLE, Psoriasis, and coeliac disease), severe Eczema, Eosinophilia, and increased level of IgE.

Methods: After DNA extraction from the patient, the high quality DNA was send for WES. The variant call format (VCF) file of WES was analyzed. The pedigree of her family was drawn and Sanger sequencing was done for suspected gene in the family.

Results: WES analysis showed the heterozygous frameshift change c.204insA (L69TfsX12) in TNFRSF13B gene (encoding the protein TACI). The pedigree of patient and Sanger sequencing of the gene showed incomplete penetration and also different clinical manifestations in patients of this family.

Conclusion: TACI have fundamental role in the B cell activation and differentiation. Although the patient did not considered Common variable immune deficiency (CVID) but about 10% of CVID patients have a mutation in TNFRSF13B. Moreover, 1%–2% of the healthy population also harbors these variants. It can be concluded that TNFRSF13B mutations maybe considered merely “disease associated” and genetic background can affect the individual health status. However, more precise analysis and functional tests are needed for genetic diagnosis.

Keywords: IEI, WES, TNFRSF13B, CVID

Flowcytometric changes of lymphocytic markers in MSMD patients: A diagnostic challenge

Maryam Asareh Zadegan Dezfouli[1], Farnoush Asareh Zadegan Dezfouli[2], Zahra Chavosh Zadeh[3]

1. Department of Allergy and clinical Immunology Shahid Beheshti Medical school Tehran Iran, Tehran, Iran.
2. Medical School of Messina, Messina Italy
3. Department of Allergy and clinical Immunology Shahid Beheshti Medical School, Tehran, Iran.

Background: MSMD are a group of innate system disorders, which, are prone to mycobacterium infections (typical and atypical), salmonella infection, and rarely other microorganisms. Various gene mutations are associated with the occurrence of this disease. Usually, after receiving the B.C.G vaccine in early infancy, we can observe B.C.G osis, which, can be very helpful in the diagnosis of these groups of diseases. However, B.C.G osis can cause morbidity and at times, mortality. In this age group, in the differential diagnosis, T-cell defects (such as SCID and Leaky SCID) can also be associated with B.C.G osis. However, with different phenotypes, can be an essential diagnostic challenge.

Methods: Since the flowcytometric changes can occur secondary to infections, interpretation of these changes makes diagnosis more difficult. We are planning to present eight patients with MSMD. All of them have a history of B.C.G osis, two cases with fungal infection, one case with Eosinophilia and two cases of brain abscess and portal vein thrombosis due to B.C.G osis complications. According to clinical presentations, Sarcoidosis seemed to be a valid diagnosis in one case. This group includes four girls and four boys with a median age of 4.4 months at the time of the development of the symptoms.

Results: Flow cytometry tests initially showed a decrease in CD3, a decrease in CD4, three cases of an inverted ratio of CD4/CD8, and, a significant increase in CD19 and CD20. Showed In two cases. According to CD3<500 and undetectable TREC, SCID was diagnosed in one case. The variants reported in the genetic studies included STAT1 (AR), RORC, IL12RB1, STAT1 (lof) AD, SPPL2A, IL12B x 3. During the treatment and the clinical course of the disease, no other symptoms were added (Except for two cases that had developed thrombosis and Brain abscesses.) and, after the treatment, the flowcytometric values were close to normal.

Conclusion: Considering the variety of genetic defects in inborn errors of immunity that sometimes have similar clinical signs, careful attention to clinical symptoms, clinical course, and long follow-up can be very useful in making decisions and determining the treatment plan. Furthermore, laboratory results must be interpreted according to the patient's clinical symptoms.

Keywords: Flowcytometry, MSMD, CD4, CD8

Global Prevalence, Mortality, and Main Characteristics of HIV-associated Pneumocystosis: A Systematic Review and Meta-Analysis

Hamid Morovati[1], Ehsan Ahmadpour[2], Hanieh Hosseini[2], Mahsa Behravan[2]

1. Shiraz University of Medical Sciences, Shiraz, Iran.
2. Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Pneumocystosis is a life-threatening HIV-associated infection. The epidemiology of this coinfection is poorly described on a worldwide scale. We conducted a systematic review and meta-analysis to evaluate the global prevalence, mortality, and main risk factors of HIV-associated pneumocystosis (HAP).

Methods: We searched related databases between January 2000 and December 2022 for studies reporting HAP. Meta-analysis was performed using StatsDirect (version 2.7.9) and STATA (version 17) according to the random-effects model for DerSimonian and Laird method and metan and metaprop commands, respectively.

Results: Our meta-analysis includes twenty-nine studies with 38554 HIV-positive, 79893 HIV-negative, and 4044 HAP populations. The pooled prevalence of HAP was estimated at 35.4% (95% CI 23.8 to 47.9). In contrast, the pooled prevalence of PcP among HIV-negative patients was 10.16% (95% CI 2 to 25.3). HIV-positive patients are almost 12 times more susceptible to PcP than the HIV-negative population (OR: 11.710; 95% CI: 5.420 to 25.297). The mortality among HAP patients was 52% higher than non-PcP patients (OR 1.522; 95% CI 0.959 to 2.416). HIV-positive men had a 7% higher chance rate for PcP than women (OR 1.073; 95% CI 0.674 to 1.706). Prophylactic (OR: 6.191; 95% CI: 0.945 to 40.545) and antiretroviral therapy (OR 3.356; 95% CI 0.785 to 14.349) were used in HAP patients six and three times more than HIV-positive PcP-negatives, respectively.

Conclusion: Despite ART and prophylactic therapy, prevalence and mortality rates of HAP remained high. Therefore, the control and management strategies should revise and updated by health policy-makers on a worldwide scale. Finally, for better management and understanding of the epidemiology and characteristics of this coinfection, designing a large-scale meta-analysis is recommended every three years.

Keywords: Pneumocystosis, HIV/AIDS, Prevalence, Mortality

Kidney and Urinary Tract Involvement in Children with Combined Immunodeficiency: A Single Referral Center Experience from Iran

Tolue Mahdavi[1], Mahnaz Jamee[2], Masoumeh Mohkam[2], Mitra Khalili[3], Seyed Erfan Rasouli[4], Solale Hosseini Gohar[6], Samin Sharafian[5], Mehrnaz Mesdaghi[6], Maryam Asarehzadegan Dezfouli[6], Maryam Heidarazad Zade[6], Zahra Chavoshzadeh[6]

1. Department of Immunology and Allergy, Rasool-E-Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
2. Pediatric Nephrology Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Radiology, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Science, Tehran, Iran
5. Department of Immunology and Allergy, Alborz University of Medical Sciences, Karaj, Iran
6. Department of Immunology and Allergy, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Combined immunodeficiencies (CIDs) are rare heterogeneous and expanding group of inborn errors of immunity (IEIs), characterized by defects in both T cell and B cell with abnormal development and/or function. The incidence rate of CID varies widely worldwide but estimated to be 1:5000 to 1:100.000 amongst live births and expected to be higher in in communities with high consanguineous marriage rates, such as Iran. The aim of this study is to investigate renal manifestation in the pediatric patients with CID. This data hopefully provides vital information to guide clinicians to help in early diagnosis, proper treatment and prevent complications

Methods: This is a prospective study on a total of 50 children with CID, at Mofid Children's Hospital, from 2021 to 2022. The laboratory evaluation included complete blood counts, serum immunoglobulin levels, measurement of antibody responses to vaccines, complement assays and flow cytometry. All patients were screened for renal function by serum bun and creatinine level. Conventional kidney ultrasound was performed to assess the size, location, detect morphologic and functional abnormalities.

Results: We reported 14 CID patients with renal disorders and 41 CID patients without renal disorders. Among patients with renal manifestations, the most common ultrasound finding was hydronephrosis (3 ,5.5%) followed by kidney stone (2 ,3.6%), bladder depris (1, 1.8%) kidney caliectasis (1, 1.8%), renal cortex echogenicity increase (1, 1.8%), cortical function abnormality (1, 1.8%), decreased corticomedullary differentiation (1, 1.8%). 30 patients (54.5%) had consanguineous parents. The level of IgG, IgM, and IgE were lower in patients with renal disorders, p=0.793, 0.750, and 0.080. CD4+ lymphocytes were lower in patients with renal disorders (p=0.756). CD3+ and CD4+ T cell lymphopenia was reported in 33.3% vs. 18.5% and 26.7% vs. 10.0% of patients with renal disorders and without it, respectively.

Conclusion: This study tried to brings to focus the relation of kidney diseases with IEI but as CID can present with syndromic features, we have not, as yet, encountered the full spectrum of variations in CID presentations.

Keywords: immunodeficiencies, inborn errors of immunity, kidney manifestation

Leukocyte adhesion deficiency

Matineh Nirouei[1]

1. Alborz University of Medical Sciences, Alborz, Iran.

Background: Leukocyte adhesion deficiency (LAD) is a rare, inherited, immunodeficiency disease caused by defects in the leukocyte adhesion process. The migration of leukocytes to the blood vessel wall needs multiple steps called adhesion cascade. In LAD, faults in rolling, integrin activation, and firm adhesion of the leukocytes have been described. Hence, LAD is categorized into 3 types, I, II, and III. LAD type I is the most common (affects the firm adhesion), while LAD type II (affects in the rolling phase) and LAD type III (affects the activation phase) are less.

Methods: In this study, we have collected 67 patients with a confirmed diagnosis of LADs from the Iranian immunodeficiency registry center. Demographic information and clinical complications were obtained from all the patients to evaluate the clinical manifestations.

Results: A total of 67 patients (38 males and 29 females) with a median age of 18 months were included in the present study. The first presentations were omphalitis in 28.35%, following delayed umbilical cord separation in 22.38% of patients. The frequency of delayed umbilical cord separation was 41.8% and was higher among other manifestations in our patients. Cellulitis and Omphalitis were in 40.3% and 38.8% of the patients, respectively.

Conclusions: We indicated in the present study that the most common clinical manifestations were delayed umbilical cord separation and recurrent infection in Iranian patients with LAD disorders. Most of the patients noted parental consanguinity, indicating that it is very important to explain families who want to marry consanguinity to a genetics consultant.

Keywords: immunodeficiency, Omphalitis, recurrent infection

Serum Interleukin-35 Level Increase in HIV/HCV Coinfection

Mohammad Jafari[1], Mostafa Hajimola Hoseini[2], Majid Khoshmirsafa[3], Maryam Keykhaee[4], Roghayeh Babaei[5], Mojgan Esparvarinha[6], Alireza Najafi[3]

1. Gerash University of Medical Sciences, Grash, Iran.
2. Immunology Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Immunology Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
4. Department of Pharmaceutical Biomaterials, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
5. Department of Medical Nanotechnology, Faculty of Advanced Sciences and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
6. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Interleukin (IL)-35 is a newly identified cytokine of the IL-12 family and a potent immunosuppressive cytokine secreted by regulatory T (Treg) cells and the regulatory B. In previous studies, the role of this cytokine in the pathogenesis of several viral diseases has been investigated. However, little information is available on the relationship between Il-35 and HIV/HCV coinfection. The aim of this study was to compare the IL-35 concentration in HIV/HCV co-infection, HIV infection, and healthy control.

Methods: A total of 50 Iranian patients under antiretroviral therapy (ART) were recruited in this study. Of these, 17 were HIV-monoinfected and 33 were HIV/HCV-coinfected. These patients were then compared to 30 HIV and HCV negative healthy controls.

Results: Our results showed that plasma levels of IL-35 had significantly elevated in HIV/HCV-coinfected patients (*$p=0.008$ and $p=0.01$*), when compared with healthy controls and HIV-monoinfected patients, respectively.

Conclusion: One of the key factors in the pathogenesis of HCV in HIV-infected individuals is the dysregulation of the immune system. The increase in IL-35 appears to play an important role here. Further studies are required to clarify such an issue.

Keywords: HIV, HIV/HCV-coinfection, Interleukin-35

Unbalanced T-cell subsets in pediatric patients with beta-thalassemia

Shideh Namazi Bayegi[1], Mehdi Shekarabi[2], Amir Ali Hamidieh[3], Maryam Behfar[3], Amene Saghazadeh[4], Mahmood Bozorgmehr[5], Nader Tajik[2], Ali Akbar Delbandi[2], Nima Rezaei[1], Samaneh Delavari[6]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
3. Pediatric Cell and Gene Therapy Research Center, Gene, Cell and Tissue Research Institute, Tehran University of Medical Sciences, Tehran, Iran
4. Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
5. Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran
6. Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Beta-thalassemia major is an autosomal recessive disorder in hemoglobin synthesis. Ineffective erythropoiesis is the main characteristic of the disease, which results in anemia following the extensive destruction of red blood cells. Chronic antigenic stimulation following frequent blood transfusions lead to immune abnormalities, especially regarding T cells, which is one of the reasons for the high susceptibility to infection in beta-thalassemia.

Methods: Six pediatric patients and six age- and sex-matched healthy children were selected. Immunophenotyping of functional T-cells was performed using flow cytometry with staining for surface and intracellular markers. The proliferative response of T lymphocytes was also investigated after labeling with CFSE and following stimulation with anti-CD3 and anti-CD28.

Results: Examination of T lymphocyte subpopulations showed a significant increase in regulatory T cells (Tregs) in beta-thalassemia patients. Hence, the Treg/Tcons (conventional T cells) and Treg/CD8 ratios were significantly increased. In addition, a significant increase in CD8 T cell proliferation activity was observed. Multivariate analysis showed a significant association of central memory cells with serum ferritin levels and the duration of transfusion. In particular, patients with cytomegalovirus (CMV) infection exhibited a significant increase in CD4 central memory cells.

Conclusion: Patients with beta-thalassemia have functionally distinct CD4 and CD8 T cell subsets imbalances, and this may contribute to their high susceptibility to infections.

Keywords: Immune abnormalities, T-cell, beta-thalassemia.

Immunometabolism

Evaluation of the relation between TIM-3/Galectin-9 axis and glutamine metabolism in AML cell lines, HL-60 and THP-1

Hooriyeh Shapourian[1], Mustafa Ghanadian[2], Nahid Eskandari[1], Abolfazl Shokouhi[3], Gülderen Yanikkaya Demirel[4], Alexandr V. Bazhin[5], Mazdak Ganjalikhani-Hakemi[6]

1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Pharmacognosy, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Endocrine and metabolism research center, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Immunology, Faculty of Medicine, Yedıtepe Unıversıty, Istanbul, Turkey
5. Department of General, Visceral and Transplant Surgery, Ludwig Maximilians University of Munich, Munich, Germany
6. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran and Department of Immunology, Faculty of Medicine, Yedıtepe Unıversıty, Istanbul, Turkey

Background: Based on evidence, T cell immunoglobulin and mucin-domain containing-3 (TIM-3) is a cell surface molecule which was first discovered on T cells. However recent studies revealed that it is also highly expressed in acute myeloid leukemia (AML) cells and it is related to AML progression. Therefore, in this study we aimed to evaluate the relation between TIM-3/Galectin-9 axis and glutamine metabolism in two types of AML cell lines, HL-60 and THP-1.

Methods: HL-60 and THP-1 cell lines were cultured in RPMI 1640 and supplemented with 10% FBS and 1% antibiotics. TIM-3 expression was induced on cells by PMA. After adding recombinant Galectin-9, RT-qPCR analysis was performed to evaluate the expression of glutaminase (GLS) and glutamate dehydrogenase (GDH) as key enzymes in glutamine metabolism pathway. Western blotting was used to detect expression of mTORC as signaling factor and also GLS protein. MTT assay was implemented to investigate the cell proliferation rate.

Results: GLS and GDH mRNA expression in HL-60 cells, at 24, 48 and 72 hours after treatment with galectin-9 had an increasing trend and the most expression of these enzymes was at 72 hours post Gal-9 treatment ($p<0.001$). However, this trend was decreasing for THP-1 cells and the most expression of these enzymes was at 24 hours after Gal-9 treatment ($p<0.001$). Western blot analysis confirmed results of RT-qPCR for GLS and mTORC expression in the two cell lines ($p<0.001$). The results of MTT assay revealed that Gal-9 could promote cell proliferation rate in both cell lines ($p<0.001$).

Conclusion: Taken together, this study suggests TIM-3/Gal-9 axis can promote glutamine metabolism in HL-60 cells and resulting to AML development. However, evaluation of this connection in THP-1 cells needs more research. Moreover, this research may develop new therapeutic approaches in future, including combinatory therapies that target TIM-3 and glutamine metabolic pathway simultaneously.

Keywords: AML, TIM-3, Galectine-9, Glutamine metabolism

Immunometabolism Meets Metabolic Reprogramming in AML: TIM-3/Gal-9 Pathway Affects Glucose and Lipid Metabolism in Acute Myeloid Leukemia Cell Lines

Mahnaz Rezaei[1], Mazdak Ganjalikhani-Hakemi[1]

1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: TIM-3 in AML cell lines can be studied from two points of view, as an immunoregulator molecule which can also affect cellular metabolism to master cell function in the concept of immunometabolism, and as an overexpressed molecule on the surface of leukemic cells and LSCs which controls the malignant transformations, including cell metabolism. In this study, we explored the effect of TIM-3 interaction with its ligand galectin-9 on the markers of glucose metabolism, lipid metabolism and resistance to oxidative stress.

Methods: HL-60 and THP-1 cell lines were cultured in appropriate condition. The expression of TIM-3 on the cell surface was ascertained by flow cytometric assay. We used real-time PCR to examine the mRNA expression of GLUT-1, HK-2, PFKFB-3, G6PD, ACC-1, ATGL and CPT-1A, colorimetric assays to measure the concentration of glucose, lactate, GSH and the enzymatic activity of G6PD, MTT assay for determination of cellular proliferation and GC-MS to designate FFAs.

Results: We observed the upregulated expression of GLUT-1, HK-2, PFKFB-3, ACC-1, CPT-1A and G6PD in the presence of Gal-9 in a time-dependent manner in both cell lines. We can also report elevation in glucose consumption, lactate release, extracellular free fatty acids (particularly palmitate and oleate) and the concentration of cellular GSH in the presence of Gal-9 as a result of its interaction with TIM-3 in both cell lines.

Conclusion: TIM-3 in AML leads leukemic cell and/or LSC through increased glycolysis, alterations in lipid metabolism and resistance to oxidative stress, all in favor of leukemic cell proliferation and survival.

Keywords: Acute Myeloid Leukemia, TIM-3, Immunometabolism, Glucose Metabolism, Lipid Metabolism

PD-L1 stimulation can promote proliferation and survival of leukemic cells by influencing glucose and fatty acid metabolism in acute myeloid leukemia

Mojdeh Soltani[1], Mustafa Ghanadian[2], Behrooz Ghezelbash[1], Abolfazl Shokouhi[3], Andrey A Zamyatnin Jr[4], Alexandr V. Bazhin[5], Mazdak Ganjalikhani-Hakemi[1]

1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Pharmacognosy, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran
3. Endocrine and metabolism research center, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Biotechnology, Sirius University of Science and Technology, Sochi, Russia
5. Department of General, Visceral and Transplant Surgery, Ludwig Maximilians University of Munich, Munich, Germany

Background: Leukemic cell metabolism has significant roles in their proliferation and survival. These metabolic adaptations may be regulated by different factors. Programmed Death Ligand -1 (CD-274) is one of immune checkpoint ligand that not only cause cancer cells immune escape, but also have some intracellular effects in these cells. PD-L1 is overexpressed on leukemic stem cells and relates with poor prognosis of AML. In this study, we investigated the effects of PD-Ll stimulation on critical metabolic pathways of glucose and fatty acid metabolisms that have important roles in proliferation and survival of leukemic cells.

Methods: After confirmation of PD-L1 expression by flow cytometry assay, we used recombinant protein PD-1 for stimulation the PD-L1 on two AML cell lines, HL-60 and THP-1. Then we examined the effect of PD-L1 stimulation on glucose and fatty acid metabolism in cells at the genomic and matabolomic levels in a time dependent manner. We investigated expression changes of rate limiting enzymes of theses metabolic pathways (G6PD, HK-2, CPT1A, ATGL1 and ACC1) by qRT-PCR and also the relative abundance changes of free fatty acids of medium by GC.

Results: We identified a correlation between PD-L1 stimulation and both fatty acid metabolism and glucose metabolism. The PD-L1 stimulated cells showed increased levels of pentose phosphate pathway and glycolysis by increasing expression of G6PD and HK-2 ($p=0.0001$). Furthermore, PD-L1 promoted fatty acid β-oxidation by increasing expression of CPT1A ($p=0.0001$), however, their fatty acids synthesis was decreased by reduction of ACC1 expression ($p=0.0001$).

Conclusion: We found that PD-L1 can promote proliferation and survival of AML stem cells probably through some metabolic changes in leukemic cells. Pentose phosphate pathway that has critical role in cell proliferation and fatty acids β-oxidation that promote cell survival, both are increased by PD-L1 stimulation on AML cells.

Keywords: Acute myeloid leukemia, AML, Fatty acid oxidation, Immunometabolism, Pentose phosphate pathway, Programmed death ligand-1, PD-1

Immunohematology and Transfusion Medicine

A cross-sectional Study of Plasma Transfusion in an Academia Affiliated Hospital in Tabriz, Iran

Roza Gharedaghi[1], Karim Shams Asenjan[2], Elham Khalaf Adeli[3], Morteza Ghojazadeh[4], Ali Akbar Pourfathollah[5]

1. Department of Immunology, Division of Hematology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Tarbiat Modares University, Faculty of Medical Science, Tehran, Iran
3. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
4. Research Center for Evidence-Based Medicine, Iranian EBM Centre: A Joanna Briggs Institute Affiliated Group, Tabriz University of Medical Sciences, Tabriz, Iran
5. Tarbiat Modares University, Faculty of Medical Science, Tehran, Iran

Background: Plasma transfusion always is not safe, and certain problems are more likely with plasma than other blood products. To reduce their side effects and improve patient clinical outcome, plasma should be administered according to specific guidelines. Estimating the frequency of blood transfusion in diseases is the first step to write guidelines. In this literature, we did it.

Methods: In this cross-sectional descriptive study, during our six-month study in the hematology and oncology hospital in Tabriz, Demographic information, laboratory tests, and an electronic investigation on received blood products were registered and bio-statistically analyzed by SPSS software.

Results: Among 450 hospitalizations, 959 plasma units was recorded for 40 patients. The Most of plasma units was administrated in patients with hemostatic disorders (595 units). Majority of administered plasma units (641 units) were recruited as plasmapheresis. Mean of plasma usage in men and women was equal (4 units). Patients below 40 years old, were the most frequently plasma recipients (median 32 units). The median of administrated plasmas in hematologic disease and solid tumors were reported 6 units and 2 units, respectively. Mean of threshold PT, PTT, INR for Plasma administration were 18.9±9.04, 37.03±12.4, 1.5±0.4, respectively. PT threshold of Plasma administration in patients with hematologic disorders and malignancies was lower than solid tumors.

Conclusion: PT, PTT, INR thresholds for plasma consumption in this center according to international standard is acceptable.

Keywords: Blood transfusion, Plasma, Thresholds

Auraptene induced cytotoxic effects in Acute Myeloid Leukemia cell lines

Majid Ghorbani[1], Fatemeh Ghorbani[2]

1. Department of Hematology and Blood Banking, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Immunology, Semnan University of Medical Sciences, Semnan, Iran

Background: Acute myeloid leukemia (AML) is one of the most commonly identified hematological malignancies with poor prognosis. This research was planned to identify the cytotoxic effects of Auraptene on HL60 and U937 cell lines.

Methods: The cytotoxic effects of Auraptene were measured by Alamar Blue assay (Resazurin) after 24 and 48 hours treatments with different doses of Auraptene. The inductive effects of Auraptene on cellular oxidative stress were investigated by determining cellular ROS levels. The cell cycle progression and cell apoptosis were also evaluated by flow cytometry method.

Results: Our findings revealed that Auraptene decreased HL60 and U937 cellular proliferation by downregulation of Cyclin D1. Auraptene also induces cellular oxidative stress by upregulation of cellular ROS levels. Auraptene induces cell cycle arrest the early and late phases of apoptosis by upregulation of Bax and p53 proteins.

Conclusion: Our data suggest that the anti-tumor function of Auraptene can be mediated by promoting apoptosis and cell cycle arrest and inducing cellular oxidative stress in HL60 and U937 cell lines. These results support that Auraptene may be used as a potent anti-tumor agent against hematologic malignancies in the further studies.

Keywords: Auraptene, AML, Cytotoxic effect, Apoptosis, Cell cycle

Identification of microRNAs involved in P53 signaling pathway in in HTLV-1 associated Adults T cell leukemia lymphoma (ATLL)

Zeynab Asgari[1]

1. Departmrent of Immunology, Kerman University of Medical Science, Kerman, Iran.

Background: HTLV-1 is the etiological cause of adult T cell leukemia-lymphoma (ATLL), a fatal malignancy of CD4+ T cells with a considerable poor prognosis, and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). MicroRNAs (miRNAs) are a group of noncoding, functional RNAs that resulting in target mRNA being degraded or suppressed during translation. It is now well understood that microRNAs are implicated in development of HTLV-1 related disorders. This study, revealed the highly important miRNAs in P53 signaling pathway expressed in ATLL patients.

Methods: The GSE31629 dataset was obtained with the GPL7731 platform. The dataset contained miRNA samples from peripheral blood mononuclear cells (PBMCs) and CD4+ T cells from 40 ATLL patients and 22 healthy donors. Adjusted $p\text{-}value < 0.05$ was determined as the threshold for DEM detection. Initial pathway analysis was performed using the Diana-mirPath web server. P53 was used as a criterion for choosing miRNAs that have a higher probability of contributing to the immunopathogenesis of ATLL.

Results: MicroRNAs were obtained by analyzing the selected DEMs with log FC <0.05 in the Diana database, the effective microRNAs in the P53 pathway implicated in the immunopathogenesis of HTLV-1 were let-7f-5p, let-7a-5p.

Discussion and conclusions: The examined microRNAs have gene targets that can be identified and measured as potential targets for the development of diagnostic, therapeutic, and preventive strategies. However, further studies are recommended to better understand the possible applications of miRNAs and their relationship with the pathogenesis of ATLL.

Keywords: Adults T cell leukemia lymphoma (ATLL), The human T-lymphotropic virus type 1(HTLV-1), microRNA, P53 signaling pathway

Impact of intravenous immunoglobulin in G6PDD neonates with hyperbilirubinemia

Seyedeh Zohreh jalali[1], Farshid Saadat[2]

1. Department of Neonatology, School of Medicine, Guilan University of Medical Sciences Rasht, Guilan, Iran.
2. Department of Immunology, School of Medicine, Guilan University of Medical Sciences Rasht, Guilan, Iran

Background: Glucose-6-phosphate dehydrogenase deficiency (G6PDD) alone or concomitant with ABO isoimmunisation is a widespread indication for neonatal exchange transfusion. The present study aimed to evaluate intravenous immunoglobulin (IVIG) in isolated G6PD deficiency neonates with hyperbilirubinemia.

Methods: In this retrospective cross-sectional study, the medical records of neonates admitted to the neonatal intensive care unit for hyperbilirubinemia due to G6PDD were reviewed. The duration of phototherapy, total bilirubin serum level, hemoglobin, Reticulocyte count and etc were evaluated. Data were analyzed with SPSS version 16

Results: Of 598 newborns that were admitted with neonatal hyperbilirubinemia, G6PDD was present in 41in which eighteen neonates were treated with phototherapy (control group), and twenty-three were maintained in phototherapy plus IVIG. There was no difference in the demographic characteristics between the two groups. The mean length of hospitalization in control group compared to IVIG+ phototherapy group was 3.28 ± 0.462 days versus 3.22 ± 0.998 days, respectively that wasn't statistically significant (*p=0.799*).

Conclusion: This study revealed that the administration of intravenous immunoglobulin in G6PDD neonates with hyperbilirubinemia should be considered as an early intervention to reduce need of exchange transfusion, although further studies are required.

Keywords: Hyperbilirubinemia, neonates, G6PDD, intravenous immunoglobulin

Prevalence and Phylogenetic Studies of Human Parvovirus 4 among Healthy Blood Donors and Deferred Donors Suspected of having HTLV-I/II Infections in Iran

Hooman Ramezany[1], Maryam Kheirandish[1], Zohreh Sharifi[1], Shahram Samiee[1], Maryam Zadsar[1]

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

Background: Human parvovirus 4 (PARV4) is an emerging virus that may infect individuals with other blood-borne viruses; despite the absence of robust and consistent symptoms, PARV4 has been linked to a wide range of clinical manifestations. Blood products primarily transmit PARV4 infection, but limited studies have been conducted on PARV4 prevalence among blood donors in Iran. This study aimed to estimate the seroprevalence (HTLV I&II total Abs), viral load (DNA), and genotyping of PARV4 in deferred blood donors suspected of having HTLV-I/II in Iran.

Methods: 230 samples of blood donors were collected during the winter and spring of 2019. To determine the HTLV-I/II antibody seroprevalence in 115 deferred blood donors, an enzyme-linked immunosorbent assay (ELIZA) has been done. Likewise, as a confirmatory test, the western blot analysis was performed on repeated HTLV-I/II ELIZA-positive samples. PARV4-PCR assays were run to estimate the prevalence of PARV4 in confirmed positive blood. The phylogenetic study on PARV4-infected donor samples was conducted following nested PCR product sequencing by Applied Biosystems. Viral load was measured using a real-time PCR test.

Results: Anti-HTLV-I/II antibodies were confirmed in 78.3% (90/115) of ELIZA-positive samples by western blot assay. The overall prevalence of PARV4 infection was 14.4% (95 % CI, 7.1 – 21.6), with a significant difference between males and females ($p<0.001$). The PARV4 infection was associated with a history of blood donation ($p<0.038$). There was no PARV4 viremia in 115 healthy blood donors. Notably, there is a strong correlation between PARV4-DNA and HTLV-I/II ELIZA-positive deferred donors ($p<0.001$). Phylogenetic analyses in sequences indicated that positive samples were comparable to genotype 2. The qPCR technique measured a high PARV4 viral load between 10^4- 10^6 DNA copies/mL in positive samples.

Conclusion: Although improved donor selection and laboratory screening can significantly reduce the prevalence of HTLV I/II infection in blood donors, Khorasan is still an endemic area of HTLV I/II infection in Iran. For the first time, we evaluated the co-infection rate of HTLV-I/II and PARV4 viruses in Iranian blood donors. We detected a high titer of PARV4-DNA among HTLV-I/II positive donors. Although this study found no evidence of PARV4 DNA in healthy blood donors, a more comprehensive statistical analysis is recommended. PARV4 is resistant to inactivation strategies applied in blood transfusion, and its inactivation has not yet been addressed. Therefore, this infection can still be a cause for concern.

Keywords: Human parvovirus 4; human T-lymphotropic virus I/II; Blood donors; co-infection; Real- 53 time PCR; phylogenetics studies

The seroprevalence and phylogenetic studies of Human Parvovirus4 in healthy blood donors and suspected of having HTLV-I/II infections in Iran

Hooman Ramezany[1], Maryam Kheirandish[2], Niloufar Kazemi Babaahmadi[3], Khadijeh Golzadeh[4]

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

Background: Human parvovirus 4 (PARV4) is an emerging virus that may infect individuals with other blood-borne viruses; despite the absence of robust and consistent symptoms, PARV4 has been linked to a wide range of clinical manifestations. Blood products primarily transmit PARV4 infection, but there are limited studies have been conducted related to PARV4 infection among blood donors in Iran. This study aimed to estimate the seroprevalence (anti- HTLV-I/II IgM and IgG), viral load (DNA), and genotyping of PARV4 in deferred blood donors suspected of having HTLV-I/II in Iran.

Methods: 230 samples of blood donors were collected during the winter and spring of 2019. To determine the HTLV-I/II antibody seroprevalence in 115 deferred blood donors enzyme-linked immunosorbent assay (ELISA) was used. For confirmatory, the western blot analysis was performed on HTLV-I/II-ELISA positive samples. Samples were tested using the PARV4-PCR assays. The phylogenetic study on PARV4 infected samples was conducted following nested PCR product sequencing. Viral load was measured using a real-time PCR test.

Results: Anti-HTLV-I/II antibodies were confirmed in 78.3% (90/115) of ELISA positive samples by western blot assay. 6.5% (15/230) of donors were positive in PARV4 real-time PCR; only 1.3% (3/230) of the samples were infected with PARV4 DNA by nested PCR. Phylogenetic analyses in sequences indicated that positive samples were comparable to genotype 2. The data revealed no correlation between HTLV-I/II infection and PARV4 viremia *($p>0.05$)*. No relationship was observed statistically between the age and sex of donors and the type of donation with PARV4 infection (*$p>0.05$*).

Conclusion: Our data demonstrated the low seroprevalence of PARV4 in HTLV-I/II infected blood donors. Based on the absence of PARV4 infection in healthy blood donors, more extensive statistical population studies are recommended, along with antibody screening.

Keywords: Human parvovirus 4, human T-lymphotropic virus I/II, Real-time PCR, Nested PCR, Blood donors, Genotyping

Toll-like receptor 4, 2 and interleukin 1 receptor associated kinase4: Possible diagnostic biomarkers in myelodysplastic syndrome patients

Parvin Khalilian[1]

1. 1Department of Immunology Faculty of Medicine Isfahan University of medical sciences. 2. Applied Physiology Research Center, Isfahan Cardiovascular Research Institute, Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Background: Myelodysplastic syndrome (MDS) is a clonal hematologic disorder which requires the integration of morphologic, cytogenetic, hematologic, and clinical findings for a successful diagnosis. Trying to find ancillary tests such as biomarkers improves the diagnosis process. Several studies showed that a disordered immune system is associated with MDS. The chronic activated innate immune system particularly Toll-like receptors (TLRs) pathway could be involved in inflammation induction. In the present study, we investigated the expression level of TLR2, TLR4, and IRAK4 in bone marrow cells of MDS patients, healthy donors, and some leukemia patients. We assessed potential capacity of TLR2, TLR4, and IRAK4 expression as biomarker to discriminate MDS from other hematologic disorders.

Methods: Patients Characteristics: in the present study we investigated the expression of TLR4, TLR2, and IRAK4 in bone marrow (BM) of MDS patients, leukemia group, and healthy group. For this purpose, we assessed the expression of TLR4, TLR2, and IRAK4 by real time-PCR.

Results: In line with new findings, we demonstrated that expression of TLR4, TLR2, and IRAK4 significantly increased in MDS BM compared to healthy group. Moreover, IRAK4 expression was raised significantly in MDS patients compared with other studied hematologic neoplasms. Also, expression levels of TLR4 and TLR2 significantly increased in MDS vs some studied non-MDS malignancies ($p<0.05$). Receiver operating characteristics (ROC) analysis and area under the curve (AUC) suggested expression of TLR4, IRAK4, and TLR2 (AUC= 0.75, AUC= 0.682, and AUC=0.702, respectively) had acceptable diagnostic values to identify MDS from other under-studied leukemia.

Conclusion: The expression of TLR2, TLR4, and IRAK4 is upregulated in MDS BM compared to healthy BM. It suggests that the expression of TLR2, TLR4, and IRAK4 could be potential biomarkers for discrimination MDS from some types of leukemia.

Keywords: Myelodysplastic syndrome, TLR2, TLR4, IRAK4

Immunopharmacology and Herbal Medicine

Alteration of macrophage function in mice treated with hydroalcoholic extract of sumac

Mahtab pourkamalzadeh[1], Seyyed Meysam Abtahi Froushani[1], Elham Naji Zavareh[1]

1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Iran.

Background: Sumac (Rhus coriaria) is one of the well-known food spices with medicinal properties. Sumac has a wide range of active pharmacological compounds with strong antioxidant activity, including anthocyanins, tannins, and flavonoids. This survey was set out to evaluate the effects of the hydroalcoholic extract of sumac on the peritoneal macrophage of mice that received it.

Methods: Male NMRI mice were randomly divided into the following five groups (n=5): Phosphate-buffered saline (PBS)-treated mice, hydroalcoholic extract of sumac -treated mice (40 mg/kg, P.O., 30 consecutive days). At the end of this step, the resident macrophages in the peritoneal cavity of mice were isolated by injecting ice-cold PBS. The macrophages were incubated with 0.1% NBT and 100 ng/ml tetradecanoylphorbol acetate for 20 minutes to assess the potential for respiratory burst evaulation. The cells were primed with LPS (10 pg/mL) for 24 h to monitor the potential of nitric oxide production. The collected supernatant was also used to evaluate the levels of cytokines IL-10 and IL-12 via ELISA. The cells were also pulsed with neutral red-stained, heat-stabilized, zymosan suspension at a 1:10 ratio for 30 minutes to assess the phagocytic ability of macrophages.

Results: The administration of hydroalcoholic extract of sumac decreased macrophages' respiratory burst, nitric oxide, and IL-12 production while increasing their IL-10 production. Macrophages isolated from rats that received an extract of sumac had lower phagocytic potential than those isolated from untreated mice.

Conclusion: It seems that the use of sumac hydroalcoholic extract inhibits the inflammatory functions of macrophages.

Keywords: Sumac; Macrophage; Phagocytosis; Respiratory burst.

Ameliorative effects of silver and zinc and gold nanoparticles synthesized using the aqueous extract of Allium saralicum on ulcerative colitis in rats

Navid Etemadi[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[2], Naser Abbasi[2]

1. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.
2. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.

Background: Medicinal plants have always been considered as worthy alternatives to chemical drugs due to their ease of access, reduction of side effects, and reasonable prices, and they have been especially noticed by researchers in the last few decades. Ulcerative colitis is a chronic inflammatory disease of the large intestine that, although the course of the disease is long, does not cause death. It can be controlled with proper treatment and diet.

Methods: forty eight male Wistar rats were divided into six groups: untreated control, positive control group (acetic acid-induced ulcerative colitis), the aqueous extract of Allium saralicum treated group (200mg/kg/day) , the silver nanoparticles (AgNPs) of Allium saralicum (0/5mg/kg/day) , the zinc nanoparticles (ZnNPs) of Allium saralicum (0/5mg/kg/day) ,the gold nanoparticles (AuNPs) of Allium saralicum (0/5mg/kg/day) and treated group with prednisolone(6mg/kg/day). After 10 consecutive days. The rats were dissected and blood, intestine samples of them collected for hematological, biochemical, Immunology, and gross parameters analysis. The data were analyzed using one-way ANOVA followed by Duncan post hoc test.

Results: The results showed that the reduction of tissue myeloperoxidase, Nitric oxide, Malondialdehyde production in gold nanoparticles (AuNPs) of Allium saralicum group compared to peridenozolone was statistically significant does not have. The gold nanoparticles (AuNPs) of aqueous extract Allium saralicum could significantly ($p \leq 0.05$) decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the Other groups. Also, the gold nanoparticles (AuNPs) of aqueous extract Allium saralicum prevented significantly ($p \leq 0.05$) small, medium and large intestine ulcers as compared to the other groups.

Conclusion: These results demonstrated the gold nanoparticles (Au NPs) of aqueous extract Allium saralicum as Suitable chemical composition is a promising strategy to improve the inflammation in a rat model of ulcerative colitis.

Keywords: gold nanoparticles (Au NPs) of aqueous extract Allium saralicum, ulcerative colitis, acetic acid

Anti-inflammatory Effects of Hydroalcoholic Extract of Achillea biebersteinii Flower on Complete Freund's Adjuvant-Induced Arthritis in Wistar Rats

Amirhossein Dolati-Somarin[1], Jalil Tavakkol-Afshari[1]

1. Department of Immunology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disorder that is demonstrated by synovial membrane and joints inflammation, swelling, autoantibody production, joints and cartilage damage, and bone erosion. Despite several therapeutic medications, severe forms of RA in many patients lead to disability. Therefore, more effective pharmacological interventions with few or no side effects, are required for RA treatment. Herbal medicines are the paramount candidate that have been investigated for a long time to find medications for RA. Achillea biebersteinii (A. biebersteinii) is the Iranian endemic plants. The medicinal properties of Achillea biebersteinii are due to the phenolic and polyphenolic components. However, the protective effects of A. biebersteinii against Complete Freund's adjuvant (CFA)-induced arthritis in rate have not yet been investigated. In the present study the anti-inflammatory activities of the hydroalcoholic extract of A. biebersteinii Flower on CFA-induced Arthritis in Wistar Rats, was evaluated.

Methods: Arthritis was induced in the Wistar rats by a one-time subplantar injection of the right hind paw with 100 µl CFA.The study groups included 48 rats, were randomly allocated to six groups: Sham, Positive Control (CFA+2 mg/kg MTX), Negetive Control (CFA), Treatment groups (CFA+100, 200, 400 mg/kg A. biebersteinii extract). A. biebersteinii extract were orally administered from day five after the CFA injection and continued for 23 days. CFA-induced inflammatory paw edema, body weight and arthritic index were evaluated for the assessment of disease progression. Moreover, the expression of IFN-γ, IL-17 and TGF-β genes were evaluated in splenic leukocytes by using Real-Time PCR technique.

Results: The results of present study revealed that A. biebersteinii extract prevent development or ameliorate arthritis symptoms. Oral administration of hydroalcoholic extract of A. biebersteinii significantly decreased paw edema and arthritis score. The expression of IFN-γ and IL-17 genes were lower in treatment groups than control group. A significant decrease in IFN-γ gene expression occurred in treatment group at the dose of 400 mg/kg.

Conclusion: To the extent of our knowledge, this is the first report about the anti-inflammatory activity of A. biebersteinii extracts on arthritis animal model. Hydroalcoholic extract of A. biebersteinii flowers appears to be promising as anti-arthritis medication, but a larger and more detailed preclinical and clinical studies especially in human is recommended.

Keywords: Rheumatoid Arthritis; Anti-inflammatory; Complete Freund's adjuvant (CFA); Achillea biebersteinii

Anti-inflammatory potential of Quercetin in COVID-19 treatment

Ali Saeedi-Boroujeni[1], Mohammad-Reza Mahmoudian-Sani[2]

1. Abadan School of Medical Sciences, Abadan, Iran.
2. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Background: SARS-CoV-2 is a betacoronavirus causing severe inflammatory pneumonia, so that excessive inflammation is considered a risk factor for the disease. According to reports, cytokine storm is strongly responsible for death in such patients. Some of the consequences of severe inflammation and cytokine storms include acute respiratory distress syndrome, acute lung injury, and multiple organ dysfunction syndromes. Phylogenetic findings show more similarity of the SARS-CoV-2 virus with bat coronaviruses, and less with SARS-CoV.

Methods: The search in the extensive literature of peer-reviewed articles published from the inception to December 2021 was conducted to identify the relevant studies, using the electronic databases of MEDLINE/PubMed, Embase, Scopus, the Cochrane Library, and the Web of Science.

Results: Quercetin is a carbohydrate-free flavonoid that is the most abundant flavonoid in vegetables and fruits and has been the most studied to determine the biological effects of flavonoids. Inflammasomes are cytosolic multi-protein complexes assembling in response to cytosolic PAMP and DAMPs, whose function is to generate active forms of cytokines IL-1β and IL-18. Activation or inhibition of the NLRP3 inflammasome is affected by regulators such as TXNIP, SIRT1 and NRF2. Quercetin suppresses the NLRP3 inflammasome by affecting these regulators.

Conclusion: Quercetin, as an anti-inflammatory, antioxidant, analgesic and inflammatory compound, is probably a potential treatment for severe inflammation and one of the main life-threatening conditions in patients with COVID-19.

Keywords: COVID-19, NLRP3 inflammasome, Quercetin, SARS-CoV-2, SIRT1, TXNIP.

Aspartame as a safe compound to mice immune system

Bamdad Riahi-Zanjani[1]

1. Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: The aim of the present study was to investigate immunotoxic effect of aspartame (ASP), as an important artificial sweetener, using BALB/c mice.

Methods: ASP was administered orally at doses of 400 and 2000 mg/kg for 15 days. Hystopathological examination of spleen and bone marrow, delayed type of hypersensitivity (DTH) response, hemagglutination titer (HA), cytokine production and lymphocyte proliferation assay were studied in various groups of animals.

Results: ASP at all doses could not produce any significant changes in hematological parameters, HA titer, DTH and lymphoproliferation responses, as well as in release of cytokines by isolated splenocytes ($p > 0.05$). Therefore, ASP did not induce any marked effects in immune system parameters of mice.

Conclusion: Finally, in current study, ASP at selected doses was found to be safe to mice immune system.

Keywords: Aspartame, immune system, DTH, HA

Comparative Effect of gold and zinc Nanoparticles of Allium saralicum and Aqueous Extract of Allium saralicum against Acetaminophen-induced Hepatotoxicity in Mice

Mohammadmehdi Zangeneh[1], Samira Zand[2], Akram Zangeneh[2]

1. Biotechnology and Medicinal Plants Research Center Ilam University of Medical Sciences, Ilam, Iran
2. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Background: Acetaminophen which is an analgesic drug causes a potentially fatal, hepatic injury when taken in overdose. Recent research have revealed gold nanoparticles' anti-toxic properties against hepatoxicity. Allium saralicum extract significantly improved acetaminophen-induced hepatic failure. In the present study we investigated the effects of gold and zinc nanoparticles and aqueous extract of Allium saralicum combination on hepatotoxicity induced in mice by acetaminophen.

Methods: 48 mice in six groups were treated by gavage as follows: groups 1 and 2 received normal saline, groups 3 received 200 mg/kg of A. saralicum hydro-alcoholic extract. Group 4 was treated with gold and zinc nanoparticles of A. saralicum 0.5 mg/kg. After 30 days, the therapeutic groups, as well as group 2, were administered a single dose of acetaminophen (500 g/kg). After 48 hours, the animals were anesthetized, and blood and liver samples were collected for histological and biochemical examinations.

Results: gold and zinc nanoparticles of A. saralicum could significantly decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, glucose, and increase HDL, total protein, albumin compared to the other treatment groups. Further, the nanoparticles of A. saralicum reduced NO, MPO, and MDA levels in plasma compared to untreated groups and A. saralicum group as well.

Conclusion: The acquired results showed the hepatoprotective potential of the aqueous extract of A. saralicum and nanoparticles combination.

Keywords: Allium saralicum, Gold and Zinc Nanoparticles, Hepatotoxicity, Acetaminophen

Comparative Effect of gold Nanoparticles of Matricaria chamomilla Aqueous Extract against Acetaminophen-induced Hepatotoxicity in Mouse

Naser Abbasi[1], Navid Etemadi[2], Mohammad Mehdi Zangeneh[1], Akram Zangeneh[1]

1. Biotechnology and Medicinal Plants Research Center Ilam University of Medical Sciences, Ilam, Iran.
2. Veterinary Graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.

Background: higher doses may lead to toxicity, including liver toxicity and liver failure. Matricaria chamomilla is a plant with flavonoid, alkaloid, and antioxidant properties.

Methods: The successful nanoparticle formation was monitored by UV–Vis spectrophotometer through color conversion due to surface plasma resonance bands at 440 nm. In this study, 32 adult male mice were divided into four groups. In group 1 as the control group, Physiological serum was given for 30 days. In group 2, acetaminophen (500 mg/kg) was given on day 29. In group 3, acetaminophen was given according to group 2, and aqueous extract of Matricaria chamomilla was given for 30 day. In group 4, acetaminophen was given according to group 2, and gold nanoparticles of were given for 30 day. At the end of Matricaria chamomilla the study period, the animal was dissected, and blood and liver samples were collected to evaluate hematological, biochemical, immunological, and histopathological parameters.

Results: Our findings showed that the gold nanoparticles of Matricaria chamomilla were able to notably reduce the levels of ALP, AST, ALT, GGT, cholesterol, triglyceride, total bilirubin, urea, and creatinine and also improve the levels of HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, as compared to the other treatment groups. Further, the gold nanoparticles of Matricaria chamomilla significantly reduced the rate of liver necrosis, erythrocyte congestion, and inflammation of inflammatory cells compared to the other groups. It also reduces NO, MPO, and MDA levels in plasma compared to other treatment groups.

Conclusion: gold nanoparticles of Matricaria chamomilla protect the liver against Acetaminophen-induced hepatotoxicity in mice.

Keywords: gold nanoparticles of aqueous extract Matricaria chamomilla, Hepatotoxicity, Acetaminophen

Comparative Effect of Nanoparticles of Falcaria vulgaris and Aqueous Extract of Falcaria vulgaris against Acetaminophen-induced Hepatotoxicity in Mice

Maryam Almasi[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[2], Hori Ghaneialvar[2], Seyed Meysam Abtahi Foroushani[3]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
2. Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran
3. Department of Microbiology, Veterinary Faculty, Urmia University, Urmia, Iran

Background: Acetaminophen is one of the most popular pain medications worldwide and it's Intentional or unintentional overdose can lead to liver failure.Falcaria vulgaris is a plant from the Apiaceae family and is traditionally used to treat ulcers and digestive problems.

Methods: 48 mice were randomly divided into 8 groups: control group (Physiological serum for 30 days), acetaminophen group (500 mg/kg for 29 days), aqueous extract of F.vulgaris (200 mg/kg for 30 days), Acetaminophen(500 mg/kg)and aqueous extract of F.vulgaris (200 mg/kg) group(Gavage was given for 30 days), gold nanoparticles of F.vulgaris group(200 mg/kg for 30 days), Gold nanoparticles of F.vulgaris (200 mg/kg) and acetaminophen (500 mg/kg) group(Gavage was given for 30 days), Zink nanoparticles of F.vulgaris group(200 mg/kg for 30 days), Zink nanoparticles of F.vulgaris (200 mg/kg) and acetaminophen (500 mg/kg) group(Gavage was given for 30 days). At the end, in order to evaluate histopathology, biochemical, and immunology, after anesthetizing and dissecting, the liver was removed and blood samples were taken from them.

Results: The results showed that the gold and zink nanoparticles of F.vulgaris were able to notably reduce the levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total bilirubin, urea, and creatinine and also improve the levels of HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the other treatment groups. Further, the gold and zink nanoparticles of Falcaria vulgaris significantly reduced the rate of liver necrosis, erythrocyte congestion, and inflammatory cells compared to the other groups. It also reduces NO, MPO, and MDA levels in plasma compared to other treatment groups.

Conclusion: gold and zink nanoparticles of F.vulgaris protect the liver against Acetaminophen-induced hepatotoxicity in mice.

Keywords: Hepatotoxicity, Falcaria vulgaris, Zink Nanoparticles, acetaminophen, Gold nanoparticles

Comparative Effects of Gold and Zinc Nanoparticles of Allium saralicum Aqueous Extract against Acetaminophen-induced Nephrotoxicity in Mouse

Naser Abbasi[1], Huri Ghanei Alvar[1], Akram Zangeneh[1], Mohammad Mehdi Zangeneh[1], Navid Etemadi[2]

1. Biotechnology and Medicinal Plants Research Center Ilam University of Medical Sciences, Ilam, Iran.
2. Veterinary Graduate, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Background: Due to their antioxidant and anti-inflammatory compounds, medicinal plants can protect the liver from damage caused by liver toxins. The aim of this study was to investigate the protective properties of Gold and Zinc Nanoparticles of Allium saralicum Fritsch against liver poisoning caused by carbon tetrachloride in mice.

Methods: In the current study, 40 adult male mice were divided into 5 groups (n=8). In group 1 as the control group, physiological serum was given for 30 days. In group 2, acetaminophen (500 mg/kg) was given on day 29. In group 3, acetaminophen was given according to group 2, and an aqueous extract of Allium saralicum (200 mg/kg) was given for 30 days. In group 4, acetaminophen was given according to group 2, and gold nanoparticles of Allium saralicum (5 mg/kg) were given for 30 days. In group 5, acetaminophen was given according to group 2, and zinc nanoparticles of Allium saralicum (5mg/kg) were given for 30 days. Finally, the animal was dissected, and blood and kidney samples were collected to evaluate biochemical, immunological, hematological, and histopathological parameters.

Results: The results indicated that the gold nanoparticles of Allium saralicum were able to notably decrease the levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total bilirubin, urea, and creatinine and also increase the levels of HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC compared to the other treatment groups. Besides, the gold nanoparticles of Allium saralicum considerably diminished kidney necrosis, erythrocyte congestion, and inflammation compared to the other groups. Also, gold nanoparticles decreased NO, MPO, and MDA levels in plasma compared to other treatment groups.

Conclusion: To conclude, gold nanoparticles of Allium saralicum was able to prevent the kidney against Acetaminophen-induced nephrotoxicity in mice.

Keywords: Gold and Zinc Nanoparticles of Allium saralicum Aqueous, Nephrotoxicity, Acetaminophen

Comparison of the effects of Methamphetamine, Methylphenidate and Risperidone on RAW 264.7 cells cytokine expressions

Parnian Navabi[1], Ghasem Mosayebi[1], Ali Ghazavi[1], Amir Mohammad Saeedi[1], Ali Ganji[1]

1. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran

Background: Methamphetamine (Meth) is a highly addictive psychoactive drug that is widely abused and would lead to serious health problems by affecting the immune system and causing inflammatory conditions. Despite extensive studies, the exact mechanism of methamphetamine and its detoxification drugs on the immune system remained almost unknown. The present study investigates the effect of methamphetamine (Meth) and two drugs used in methamphetamine-intoxication, including methylphenidate (MPH) and risperidone (RSP) in non-toxic concentrations, on the state of macrophage polarization.

Methods: RAW 264.7 cells with Meth, MPH and RSP were co-cultured at various concentrations for 36 h, and the viability tests were applied using MTT colorimetric assay. The quantitative reverse transcription-polymerase chain reaction was used to evaluate the gene expression of IL-6, TNF-α, INF-γ (as M1 cytokines) and IL-4, IL-10 and TGF-β (as M2 cytokines).

Results: The minimum effective cytotoxic concentration of Meth, MPH and RSP on RAW264.7 cells was determined as 250, 300 and 120 µmol/L, respectively. Findings showed that treatment of RAW264.7 cells with Meth significantly increased mRNA expression of cytokines IL-6 ($p<0.05$), TNF-α ($p<0.01$) and IFN-γ ($p<0.001$) and also led to a significant decrease in the expression of cytokines IL-4 ($p< 0.05$), IL-10 ($p< 0.05$) and TGF-β ($p< 0.001$). Meanwhile, adding the combination of RSP and MPH was able to significantly reverse the decrease in the expression of IL-4, IL-10 and TGF-β caused by Meth treatment. Also, the combination of RSP and MPH could significantly reduce the increasing inflammatory cytokines caused by Meth treatment.

Conclusions: Our study demonstrated that Meth could switch gene expression of RAW264.7 cells toward the M1-phenotype and induce inflammatory response. The combination of RSP and MPH reduced meth function by increasing the expression of anti-inflammatory cytokines and suppressing the expression of pro-inflammatory cytokines.

Keywords: Methamphetamine, Methylphenidate, Risperidone and RAW 264.7 cells

Cuscuta campestris induces apoptosis by increasing reactive oxygen species generation in human leukemic cells

Maliheh Moradzadeh[1], Azar Hoseini[2]

1. Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran.

2. Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Cuscuta campestris or common dodder is a holoparasitic plant that has been valorized for treatment of liver injury and cancer prevention in traditional medicine. Recently, extract of C. campestris had shown moderate antimicrobial properties and cytotoxic effects. In this study, we examined the level of cellular oxidants, cytotoxicity, apoptosis and differentiation induced by hydroalcoholic extract of C. campestris (CCE) (12.5-200 µg/ml), as well as arsenic trioxide (As2O3, 50 µM), in human leukemic (HL60 and NB4) and normal polymorph nuclear cells after 72 hr treatment.

Methods: Resazurin assay was used to determine cell viability following treatment with C. campestris. Intracellular reactive oxygen species (ROS) and apoptotic cells were measured by fluorimetry using carboxy 2′, 7′-dichlorofluorescein diacetate and propidium iodide (PI), as staining reagents, respectively. The differentiation of leukemic cells was evaluated by Giemsa staining and nitro blue tetrazolium (NBT) reduction.

Results: C. campestris inhibited cell viability with IC50 values of 23.9 µg/ml for HL60 and 60.3 µg/ml for NB4 cells after 72 hr treatment. ROS formation was also concentration-dependently increased following treatment with C. campestris. In addition, the number of apoptotic cells significantly increased to 88.4% and 62.3% in CCE (200 µg/ml)-treated HL60 and NB4 cells, respectively, which was higher than that of As2O3 (50 µM)-treated leukemic cells ($p<0.001$). Nonetheless, C. campestris did not induce differentiation of leukemic cells towards granulocytic pattern.

Conclusion: The present study demonstrated that C. campestris induced apoptosis through ROS production without having differential effect on leukemic cells, in concentration- and time-dependent manners. Understanding of precise signaling pathway by which C. campestris induce apoptosis, needs further research.

Keywords: Cuscuta campestris, Leukemia, Apoptosis, Differentiation, ROS

Dapagliflozin exerts anti-inflammatory effects via inhibition of LPS-induced TLR-4 overexpression and NF-κB activation in human endothelial cells and differentiated macrophages

Elaheh Abdollahi[1], Massoumeh Shafiei[2], Ali-Akbar Delbandi[3], Fariborz Keyhanfar[2], Reza Falak[2], Mansour Torab[2]

1. Department of Pharmacology and Toxicology, School of Pharmacy, Iran University of Medical Sciences, Tehran, Iran
2. Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Atherosclerosis is widely accepted as an inflammatory disease. Evidence has demonstrated that a new class of anti-diabetic drugs, sodium-glucose co-transporter type 2 (SGLT-2) inhibitors, could exert beneficial effects on atherosclerotic complications of diabetes. Hence, we aimed to assess the direct anti-inflammatory effects of the SGLT-2 inhibitor dapagliflozin (DAPA) on two cell types involved in the process of atherogenesis, i.e., endothelial cells and macrophages.

Methods: Human umbilical vein endothelial cells (HUVECs) and macrophages differentiated from human monocytes were exposed to DAPA and lipopolysaccharide (LPS 20 ng/mL) for 24 h under either normal (5.5 mmol/L, NG) or high glucose (25 mmol/L, HG) conditions. Then, values of inflammatory cytokines including TNF-α, IL-1β, IL-6 and IL-8 were determined in the cell culture medium. In addition, the expression of inflammatory markers, toll-like receptor 4 (TLR-4)/nuclear factor kappa B (NF-κB), were measured by western blot. Furthermore, levels of miR-146a and miR-155 were evaluated by quantitative reverse transcription polymerase chain reaction. We also assessed alteration in the macrophage polarization (M1/M2 ratio) in the present study.

Results: A significant rise was noticed in the inflammatory mediators in the LPS-treated control groups both under NG and HG conditions. Following 24 h incubation, DAPA could significantly attenuate LPS-induced cytokine secretion as well as NF-κB p65 phosphorylation and TLR-4 expression in HUVECS and differentiated human macrophages under both NG and HG states. DAPA (0.5 µM) could significantly attenuate TLR-4 levels (23.9% and 33.1% under NG and HG conditions in HUVECs and 53.3% and 52.4% under NG and HG states in macrophages, respectively). NF-κB p65 phosphorylation was also significantly decreased to 30.1% under NG condition in HUVECs and 51.9% and 34.5% under NG and HG states in macrophages by 0.5 µM DAPA. Under high glucose conditions in HUVECs, DAPA (0.5 µM) reduced NF-κB p65 phosphorylation level by 13%, however it didn't reach statistically significant. Moreover, DAPA elevated expression levels of anti-inflammatory miR-146a, while values of miR-155 decreased in those cells. DAPA also caused a shift from inflammatory M1 macrophages toward M2-dominant macrophages.

Conclusion: These data suggest that, regardless of glucose concentrations, DAPA could exert direct anti-inflammatory effects, at least partly, by inhibiting the expression of TLR-4/NF-κB and secretion of pro-inflammatory mediators. In addition, DAPA significantly raised miR-146a and reduced miR-155, which further confirms the anti-inflammatory effects of the drug in vitro. More research is required to explore anti-inflammatory actions of DAPA in greater detail and establish the precise underlying mechanisms of SGLT-2s inhibitors involved in the management of atherogenesis.

Keywords: Dapagliflozin, SGLT-2 inhibitor, TLR-4, NF-κB, inflammatory cytokines, HUVECs, high glucose, macrophages polarization, miR-146a, miR-155

Effect of Berberine on drug-resistance associated autophagy in leukemia cells of Chronic Lymphocytic Leukemia

Ghazaleh Alizad[1], Parviz Kokhaie[2], Davood Mozaffari[3], Ali Memarian[4]

1. Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
2. Immune and Gene Therapy Laboratory, Cancer Centre Karolinska, Department of Oncology and Pathology, Karolinska Institute, Stockholm, Sweden
3. Department of Oncology, 5-Azar Hospital, Golestan University of Medical Sciences, Gorgan, Iran
4. Stem Cell Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Background: Drug resistances and post-remission relapses that occur after treatment in B-chronic lymphocytic leukemia (B-CLL) patients force the necessity of new treatment strategies, with better clinical efficacy and fewer side effects. Since autophagy plays a key role in the apoptosis resistance of cancer cells caused by anticancer drugs, this study aims to investigate the effects of "Berberine" as a traditional therapeutic candidate on the autophagy involved in treatment resistance in B-CLL leukemic cells.

Methods: Peripheral blood mononuclear cells (PBMC) of 7 B-CLL patients and 7 healthy individuals were treated with 25µM Berberine and 2µM Idelalisib, as a standard treatment control, for 24 hours. Subsequently, the expression level of Beclin-1 (as an index of autophagy function) and HMGB-1 (as an internal control of treatment resistance) were evaluated in the CD19+ cells by flow cytometry. Cell apoptosis was also measured by flow cytometry following Berberine and Idelalisib treatment.

Results: Examination of treated leukemic B cells demonstrated that Berberine significantly decreased the level of both HMGB-1 and Beclin-1, compared to Idelalisib, while it only reduced Beclin-1 in the B cells of normal individuals. Apoptosis assay also demonstrated no toxic effect of Berberine and Idelalisib within 24 hours on the PBMC cells of patients and normal subjects.

Conclusion: Considering the anti-cancer effects of Berberine, and its efficiency on a synchronized decrease of the Beclin-1 and HMGB-1 expression vs. Idelalisib, Berberine could affect the mechanisms of autophagy and overcome therapy resistance of B-CLL patients. These findings suggest that Berberine could be employed as one of the complementary therapeutic candidates in B-CLL patients.

Keywords: chronic lymphocytic leukemia (CLL), Berberine, autophagy, drug-resistance

Effects of Menthol on in vitro proliferation and cytokine secretion of human peripheral blood mononuclear cells

Maryam Bayat[1], Kurosh Kalantar[1], Zahra Amirghofran[1]

1. Shiraz University of Medical Sciences, Shiraz, Iran.

Background: Menthol is one of the most important constituents of Mentha longifolia (19-32.5%) with various pharmacological effects. In this study, we evaluated the immunoinhibitory effects of Menthol on human peripheral blood mononuclear cells (PBMCs).

Methods: Effect of different concentration of Menthol on proliferation peripheral blood mononuclear cells was examined by BrdU assay, and cell viability was performed by propidium iodide (PI) staining using flow cytometry. Also, analysis of interferon (IFN) γ and interleukin (IL)-4 cytokine production was evaluated by ELISA in lymphocytes stimulated with phytohemagglutinin (PHA) and phorbol myristate acetate/calcium ionophore (PMA/CI).

Results: The results of BrdU assay indicated the capability of this compound to inhibit cell proliferation and activation of PBMCs in a dose-dependent manner, from 88.7% at 50 µg/ml to 3.63% at 800 µg/ml ($p<0.05$). According to the results of PI staining, this inhibitory effect was not due to cell death and apoptosis. The Effect of Menthol on the production of IL-4 and IFN-γ cytokines in PBMCs by ELISA showed that the production of IFN-γ in PHA-stimulated cells, significantly reduced from 519 pg/ml in positive control to 266 pg/ml at 400 µg/ml ($p<0.001$), and 79.6 pg/ml at 800 µg/ml ($p<0.001$). Moreover, in cells stimulated with PMA (50 ng/ml) CI (1 µg/ml) Menthol decreased the production of IFN-γ in a dose dependently manner from 331 pg/ml in positive control to 188 pg/ml at 400 µg/ml ($p<0.01$) and 36.6 pg/ml at 800 µg/ml ($p<0.001$). Therefore, both PMA/CI and PHA signaling pathways decreased IFN-γ production to more than 80% at 800 µg/ml, but had no significant effect on IL-4 release.

Conclusion: The results of this study showed the immunoinhibitory effects of Menthol on the growth of PBMCs and cytokine levels. This component has the capability to be considered as an immunomodulatory agent in inflammatory diseases.

Keywords: Immunoinhibitory, Menthol, Human lymphocytes, Cytokine production

Effects of the guluronic acid (G2013) on inflammatory components in non-alcoholic steatohepatitis patients under in vitro conditions

Safa Tahmasebi[1], Abbas Mirshafiey[2]

1. Shahid Behesti University of Medical Sciences, Tehran, Iran.
2. Tehran University of Medical Sciences, Tehran, Iran.

Background: The buildup of fatty acids in the liver causes non-alcoholic steatohepatitis (NASH). The hyper-inflammation in NASH is caused by the increased production of pro-inflammatory factors. α-L-Guluronic acid (G2013), a brand-new NSAID with immunomodulatory effects, is derived from plants. The current study looked at how G2013 affected inflammatory markers in PBMCs from NASH patients.

Methods: Both 14 healthy controls and 14 NASH patients' PBMCs were extracted and cultured. Along with the diclofenac optimal dose (3 g/mL), the patient's cells were treated with low (5 g/mL) and moderate (25 g/mL) doses of G2013. Real-time PCR and ELISA were used to measure the amounts of expression and secretion of the different variables.

Results: The results showed that when compared to healthy people, NASH patients had considerably higher levels of TLR4 and NF-B expression as well as TNF- and IL-6 cytokine release. In cells treated with low and moderate dosages of G2013, the expression levels of TLR4 and NF-B were remarkably downregulated. Both the secretion level of IL-6 and the secretion level of TNF- utilizing the low and moderate doses of G2013, respectively, were significantly reduced.

Conclusion: The findings showed that in PMBCs from NASH cases, G2013 could significantly lower the expression and secretion levels of examined factors (TLR4, NF-B, TNF-, and IL-6).We anticipate that G2013 will be a promising immunomodulatory drug in lowering inflammation and improving patients since there is now no treatment for NASH patients.

Keywords: NASH, TLR4, NF-κB, IL-6, TNF-α, α-L-guluronic acid

Evaluating the Efficacy of Althaea Officinalis Extract in Atopic Eczema Patients: a Double-blind Randomized Controlled Trial

Farahzad Jabbari-Azad[1], Roshanak Salari[2], Negar Khalighi[1], Maral Barzegar-Amini[1], Jalil Tavakkol-Afshari[3], Pouran Layegh[4]

1. Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Pharmaceutical Sciences in Persian Medicine, School of Persian and Complementary Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
4. Cutaneous Leishmaniosis Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Althaea Officinalis has been known as a recent anti-inflammatory agent that might alleviate atopic eczema symptoms through its immunomodulatory effects. In the present study, we compared the impact of steroids with the liposomal formulation of Althaea Officinalis flower extract in treating atopic eczema

Methods: We enrolled forty moderate to severe allergic atopic eczema cases confirmed by skin prick test in a double-blind randomized controlled trial phase II. As the intervention, we tested steroids and topical Althaea Officinalis extract on the left and right side of the body's lesions, respectively. Furthermore, steroids and Eucerin (as a topical placebo) were applied to the left and right side of the body's lesions in the control group. To check the alleviating effects, we assessed SCORAD (SCORing Atopic Dermatitis) at three stages: baseline, two weeks, and four weeks after the trial completion. SPSS version 22 was utilized for data analysis.

Results: The SCORAD decreased significantly in the active treatment group with both steroid and Althaea Officinalis two and four weeks after the trial *($p<0.001$).* Although a further decrease was seen in treatment with steroids, there were no significant differences between the two treatments. In the control group, the SCORAD reduced significantly with steroid treatment after two and four weeks *($p<0.001$).* The trial did not lead to any adverse reactions.

Conclusion: According to the similar effectiveness of Althaea Officinalis extract and its lower potential side effects compared with steroids, it is recommended to give thought to this anti-inflammatory agent as a new treatment option for atopic eczema cases.

Keywords: Althaea Officinalis Extract, Atopic Eczema, SCORing Atopic Dermatitis

Evaluation of Marhame-Mafasel ointment efficacy on collagen-induced arthritis rat model

Hedieh Haji Khodaverdi Khani[1], Roya Yaraee[1], Mohsen Naseri[2]

1. Department of Immunology, School of Medical Science, University of Shahed, Tehran, Iran.
2. Traditional Medicine Clinical Trial Research Center, University of Shahed, Tehran, Iran.

Background: Rheumatoid arthritis as an autoimmune disorder, primarily concerns the joints of the hands and feet and affects every patient's quality of life, which can lead to job loss, social isolation and other consequences. Because of the side effects of chemical drugs that are used in treating RA, we encourage to evaluate the efficacy of the herbal remedy called Marhame-Mafasel ointment which can be safe, cost-effective and easy to use.

Methods: After inducing CIA in Wistar rats, they were divided into three treatment groups: Marhame-Mafasel ointment, Pyroxicam gel, and a mixture of Marhame-Mafasel & Pyroxicam. We measured their body weight and foot pad volume during the trial. We also performed the chimney test at the end of the trial to assess their capacity to move and use their joints. Following animal sacrifice, we collected serum, organs such as the liver, kidney, thymus, and spleen, as well as both foot pads. We measured IL-6, TNF-, anti-CII antibody, CRP, MDA, TAC, TOC, GGT, BUN, Creatinine, AST, ALT, and ALP using serum. Furthermore, the trainer rated four categories of inflammation, synovial hyperplasia, pannus formation, and cartilage loss as 0 = normal, 1 = mild, 2 = moderate, and 3 = severe changes by providing H&E histological sections from the foot pads.

Results: When compared to the CIA group, treatment with a mixture of Marhame-Mafasel & Pyroxicam reduced paw edema, raised body weight, and enhanced motor coordination. Furthermore, when compared to the CIA group, the antibody response and CRP factors in the Marhame-Mafasel group had decreased significantly *($p<0.05$)*. Meanwhile, pathology revealed that the mixture of Marhame-Mafasel & Pyroxicam group could significantly *($p<0.05$) ($p<0.01$)* reduce inflammation, synovial hyperplasia, pannus formation, and cartilage loss in the two foot pads when compared to the CIA group.

Conclusion: The rats in the mixture of Marhame-Mafasel & Pyroxicam group were in better condition, which could indicate that combining these two ointments improved their efficacy.

Keywords: Rheumatoid arthritis, Marhame-Mafasel ointment, Collagen-induced model, anti-CII antibody

Evaluation the effects of Lactobacillus casei probiotic in combination with hydro alcoholic extract of jujube fruit on inflammatory cytokines of experimental model of ulcerative colitis

Shabnam Bahrami[1], Nahid Babaei[1], Hadi di Esmaeili Gouvarchinghaleh[2], Mahdieh Farzanehpour[2]

1. Department of Molecular Cell Biology and Genetics, Bushehr Branch, Islamic Azad University, Bushehr, Iran.
2. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: The mucosa of the colon and rectum exhibit diffuse inflammation in ulcerative colitis (UC), a chronic condition. Bloody diarrhea is the most prominent clinical sign of UC. Exacerbations and remissions, which may happen on their own or as a result of medication modifications or coexisting disorders, characterize the clinical history. The goal of the current investigation was to assess the effects of the probiotic Lactobacillus casei in combination with the hydro-alcoholic jujube fruit extract on inflammatory cytokines in an animal model of ulcerative colitis.

Methods: The Baqiyatallah University of Medical Science was the site of the current experimental study. Three male Balb/c groups were divided into the control colitis, Lactobacillus casei probiotic extract treated, hydro alcoholic extract of jujube fruit treated, and combined treatment groups. UC was generated in these animals using acetic acid. The animals were examined for gross and microscopic pathologies, inflammatory mediator production, and stress oxidative profile in the gut tissue after 10 days. Using the SPSS program, the statistical analysis was carried out (version 23.0). Statistics were judged significant at $P<0.05$.

Results: In comparison to the control colitis group, the combination treatment of Lactobacillus casei probiotic extract and hydro alcoholic jujube fruit extract significantly decreased the level of inflammatory mediators and improved the oxidative stress profile in colonic tissue ($p=0.0001$).

Conclusion: The results of the current study suggest that jujube fruit extracts and the probiotic Lactobacillus casei may be effective treatments for ulcerative colitis.

Keywords: Keywords: Lactobacillus casei, Jujube fruit, inflammatory cytokines, Ulcerative colitis

Hepatoprotective, nephroprotective, hematoprotective, immunoprotective, and gastroduodenal protective properties effects of the silver and zinc nanoparticles synthesized using the aqueous extract of Artemisia dracunculus in Wistar male rats

Seyyed Jamaleddin Tabatabaei Aghda*[1], Mohammad Mahdi Zangeneh[2], Navid Etemadi[3], Akram Zangeneh[4]

1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
2. Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran.
3. DVM, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
4. Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran.

Background: The recent studies have indicated the properties of plants and the silver and zinc nanoparticles in the prevention of gastroduodenal ulcers.Artemisia dracunculus has been used in traditional medicine as a therapeutical supplement. The aim of our research was to survey the preventive property of the silver and zinc nanoparticles synthesized using the aqueous extract of Artemisia dracunculus on ibuprofen-induced gastroduodenal ulcers by investigating the biochemical, hematological, immunological, and microscopic approaches in rats.

Methods: In this study, 48 rats were used. The animals were randomly divided into six subgroups, including negative healthy control, untreated negative control, the positive control receiving omeprazole 60 mg/kg, one group receiving the aqueous extract of Artemisia dracunculus at 180 mg/kg concentrations, and a group receiving the of silver nanoparticles (AgNPs) of aqueous extract Artemisia dracunculus at 0/5 mg/kg concentrations. a group receiving the of zinc nanoparticles (ZnNPs) of aqueous extract Artemisia dracunculus at 0/5 mg/kg concentrations. After 14 days, gastroduodenal ulcers were caused by ibuprofen 400 mg/kg. Four hours after oral administration of ibuprofen, the rats were sacrificed and blood, stomach, and duodenum samples of them collected for biochemical, hematological, immunological, and microscopic parameters analysis.

Results: The silver nanoparticles (AgNPs) of aqueous extract Artemisia dracunculus could significantly *($p \leq 0.05$)* reduce the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, creatinine, IL1, IL6, IL12, IL18, IFN-Y, and TNF-α and increase HDL, total protein, albumin, WBC, platelet, RBC, IL4, IL5, IL10, IL13, and IFN-α as compared to the other groups. Also, the silver nanoparticles (AgNPs) of aqueous extract Artemisia dracunculus prevented significantly *($p \leq 0.05$)* gastroduodenal ulcers as compared to the other groups.

Conclusion: The obtained results indicated the hepatoprotective, nephroprotective, hematoprotective, immunoprotective, and gastroduodenal protective properties of silver nanoparticles (AgNPs) of aqueous extract Artemisia dracunculus.

Keywords: silver nanoparticles (AgNPs) of aqueous extract Artemisia dracunculus, hepatoprotective, nephroprotective, hematoprotective, immunoprotectie, gastroduodenal protective

Immunomodulatory and antioxidant effects of pomegranate seed oil on treatments of osteoporosis; systematic review

Soheil Sadr[1], Hamid Reza Chaboki[1], Farideh Akbarian[1], Hossein Kazemi mehrjerdi[1]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Osteoporosis is characterized by a reduction of bone mass and destruction of bone structures, followed by high bone fragility and susceptibility. Primary osteoporosis is caused by estrogen deficiency and constitutes 95% of all cases. The pomegranate seed oil (PSO) contains 17-α-estradiol, one of the newly found phytosterols with synergistic health effects on estrogen-related physiological reactions. This study aims to evaluate the protective effects of PSO on the treatment of osteoporosis.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on the Immunomodulatory and antioxidant effects of pomegranate seed oil on treatments of osteoporosis in rats from January 2000 to January 2023. Twelve affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Estrogen modifies the production of bone-resolving cytokines such as interleukin 1 and 6, bone-stimulating factors such as colony-stimulating factor, insulin-like growth factors 1 and 2, osteoprotegerin, and the number of vitamin D receptors in bone. Hesperidin's antioxidant activity and inhibition of osteoclastic superoxide availability may help to decrease bone resorption. Another theory is that the plant metabolite acts on bone cells via estrogen receptors. Estrogen receptors are found in bone marrow osteoblasts and stromal cells. The primary steroidal estrogen in pomegranate seed oil has been identified as 17-alpha-estradiol, a biosimilar to estrogen that is less dangerous than other forms of estrogen.

Conclusion: According to the findings of this research, PSO reduced the pathological effects of osteoporosis in the bones of ovariectomized rats. It can be inferred that it is beneficial for postmenopausal women undergoing estrogen replacement therapy.

Keywords: Estradiol, Histopathology, Osteoporosis, Pomegranate

Immunomodulatory effects of aqueous extract of Narcissus tazetta after subacute exposure to mice: A tiered-approach immunotoxicity screening

Bamdad Riahi-Zanjani[1]

1. Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Narcissus tazetta is a perennial herbaceous plant with a history of pharmacologic and biochemical effects. In the present study, the immunodulatory effects of aqueous extract of Narcissus tazetta (AENT) were investigated in BALB/c mice.

Methods: AENT was administered orally at doses of 25 and 50 mg/kg for 14 consecutive days. Following the exposure, host hematological parameters, spleen cellularity and histopathology, as well as delayed-type hypersensitivity (DTH) responses, hemagglutination titers (HA), splenocyte cytokine production and lymphocyte proliferation were studied in all of the test groups of animals. The results showed that high dose of AET (50 mg/kg) could stimulate both cellular and humoral immune functions in the treated hosts. In addition, AENT at 50 mg/kg appeared to impact on lymphoproliferation assay.

Results: Based on the finding here, it would seem that AENT has effective immunostimulant properties. Mechanistic studies are required to determine exactly how this material is acting to impart the immunostimulatory effects demonstrated here.

Conclusion: At the same time, further research should also be performed on AENT to further develop its potential use as an effective immunostimulant or co-adjuvant for the treatment of diseases caused by a weakened or unwanted immune response.

Keywords: Narcissus tazetta, Immunomodulatory, Cellular immunity, Humoral immunity

Immunomodulatory effects of aqueous extract of Tarragon after subacute exposure to mice: A tiered-approach immunotoxicity screening

Bamdad Riahi-Zanjani[1]

1. Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Tarragon with scientific name of Artemisia dracunculus is a perennial herbaceous plant with a wide range of pharmacologic and biochemical effects. In the present study, the immunodulatory effects of aqueous extract of tarragon (AET) were investigated in BALB/c mice.

Methods: AET was administered orally at doses of 250 and 500 mg/kg for 14 consecutive days. Following the exposure, host hematological parameters, spleen cellularity and histopathology, as well as delayed-type hypersensitivity (DTH) responses, hemagglutination titers (HA), splenocyte cytokine production and lymphocyte proliferation were studied in all of the test groups of animals. The results showed that both doses of AET (250 and 500 mg/kg) could stimulate both cellular and humoral immune functions in the treated hosts. In addition, AET at 500 mg/kg appeared to impact on lymphoproliferation assay.

Results: Based on the finding here, it would seem that AET has effective immunostimulant properties. Mechanistic studies are required to determine exactly how this material is acting to impart the immunostimulatory effects demonstrated here.

Conclusion: At the same time, further research should also be performed on tarragon to further develop its potential use as an effective immunostimulant or co-adjuvant for the treatment of diseases caused by a weakened or unwanted immune response.

Keywords: Tarragon, Immunomodulatory, Cellular immunity, Humoral immunity

Immunomodulatory effects of Menthol on IFN-γ production and gene expression of T cell subsets transcription factors

Maryam Bayat[1], Kurosh Kalantar[1], Zahra Amirghofran[1]

1. Shiraz University of Medical Sciences, Shiraz, Iran.

Background: Immunomodulation refers to the manipulation of any part of the immune response, as a therapeutic interruption. In this study, we analyzed the immunomodulatory effects of Menthol, main component of Mentha longifolia, on IFN-γ production and gene expression of T cell subsets transcription factors in human lymphocytes.

Methods: The percentage of IFN-γ producing TCD4+ cells were evaluated by flow cytometry after 24h and 48h stimulation with PHA and exposure to brefeldin A, for 5 and 16 h. Also, investigated after 5h stimulation with PMA/CI in the presence of various concentrations of Menthol. To assessment of T helper (Th) cells transcription factors, PBMCs stimulated with PHA and treated by Menthol (100-800 µg/ml) for 24 h. Then, RNA extracted, cDNA synthesized, and gene expressions measured using real time-PCR.

Results: The results of flow cytometry indicated that Menthol in 24 h and 48 h PHA-stimulated cells with blocking 5h reduced dose dependently the number of CD4+IFNγ+ cells to 2.53% *($p<0.05$*) and 1.60% *($p<0.001$)*, respectively. Furthermore, in 24 h and 48 h PHA-stimulated cells with blocking 16 h, Menthol diminished these cells to 2.40% *($p<0.05$)* and 3.98% *($p<0.001$*), respectively. Additionally, in PMA/CI-stimulated cells the number of CD4+ IFNγ+ cells decreased to 10.4 % at 800 µg/ml compared with positive control. Menthol dose dependently decreased gene expression levels of T-bet (2.76 RFC), ROR-γt (0.76 RFC), and Foxp-3 (1.46 RFC) ($p<0.001$) in the highest concentration, but had no effect on GATA3. Also, the ratio of T-bet/GATA3, T-bet/Foxp3 and ROR-γt/Foxp3 was dose-dependently decreased.

Conclusion: Menthol reduced the number of IFN-γ-expressing CD4+T cells, and significantly down-regulated the expression of transcription factors specific for T cell subsets. Thus, this effective component could be considered as a suitable candidate for use in inflammatory diseases mediated by Th1 cells.

Keywords: Menthol, Immunomodulation, gene expression, IFN-γ production

Improving effects of gold and copper nanoparticles on synthesized using aqueous extract of Falcaria vulgaris on acetic acid-induced ulcerative colitis in rats

Mohammad Mahdi Zangeneh[1], Seyyed Jamaleddin Tabatabaei Aghda[2], Akram Zangeneh[3], Navid Etemadi[4]

1. Biotechnology and medicinal plants Research center, Ilam University of Medical Sciences, Ilam, Iran.
2. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
3. Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran.
4. DVM, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Background: Ulcerative colitis (UC), a subcategory of inflammatory bowel disease, afflicts 1-2 million people in the United States, and many more worldwide. Despite advances in treatment, only approximately 40% of patients achieve clinical remission at the end of a year. This study was designed to evaluate the effects of gold and copper nanoparticles of Falcaria vulgaris on ulcerative colitis in rats.

Methods: forty male Wistar rats were divided into five groups: untreated control, positive control group (acetic acid-induced ulcerative colitis), the aqueous extract of Falcaria vulgaris treated group (200mg/kg/day), the copper nanoparticles of Falcaria vulgaris (0/5mg/kg/day), the gold nanoparticles of Falcaria vulgaris (0/5mg/kg/day) and treated group with prednisolone (6mg/kg/day). After 10 consecutives days.the rats were dissected and blood, intestine samples of them collected for hematological, biochemical, Immunology, gross parameters analysis and Macroscopic examination of the lesion site. The data were analyzed using one-way ANOVA followed by Duncan post hoc test.

Results: The results showed that the reduction of tissue myeloperoxidase, Nitric oxide, Malondialdehyde production in gold nanoparticles of group compared to peridenozolone was statistically significant does not have. The gold nanoparticles of Falcaria vulgaris aqueous extract could significantly *($p \leq 0.05$)* decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the other groups. Also, gold nanoparticles of Falcaria vulgaris aqueous extract prevented significantly *($p \leq 0.05$)* small, medium and large intestine ulcers as compared to the other groups.

Conclusion: These results demonstrated gold nanoparticles of Falcaria vulgaris aqueous extract may be a promising agent to ameliorate ulcerative colitis.

Keywords: gold and copper nanoparticles of Falcaria vulgaris aqueous extract, acetic acid, ulcerative colitis, wistar rat

In vitro and in vivo Anti-inflammatory and Antidiabetic Properties of Phenolic Antioxidant from Camellia sinensis

Fateme Ahmadzadeh[1], Saeid Abedian[1]

1. Department of Immunology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

Background: Epigallocatechin-3-gallate (EGCG), the major catechin derived from the leaves of the plant Camellia sinensis also known as tea tree, has a pleiotropic mode of action on various cell types. In this study, we investigated the concentration-dependent effect of EGCG on proliferation and secretory function of phytohemagglutinin (PHA)-stimulated mouse spleen cells and pancreatic beta cell line (βTC-3) in vitro in relation to gene expression of transcription factors in vivo.

Methods: Type 1 diabetes was induced in C57BL/6 by multiple injection of streptozotocin (STZ). The isolated diabetic splenocytes -stimulated with PHA- and STZ-treated βTC-3 were cultured in the presence of graded concentrations of EGCG and unstimulated cells served as control. Proliferation, IFN-γ and IL-10 production and insulin secretion were evaluated in coculture experiments by MTT assay and ELISA, respectively. In addition, EGCG was intraperitoneally administrated to diabetic mice at the doses of 25 and 50 mg/kg and gene expression of transcription factors T-bet, GATA-3, foxp-3 and insulin were assessed using real-time PCR. Histopathology was used for the assessment of pancreatic islets using H&E staining.

Results: The data showed that EGCG ameliorated diabetes by decreasing inflammation and STZ-induced damage, accompanied by decreased splenocyte T cell proliferative response and reduced inflammatory cytokine in higher doses. The effect of EGCG was attributable to its down-regulation of the expressions of T-bet and up-regulation of the expressions of GATA-3, foxp-3. Moreover, EGCG protected βTC-3 cells against STZ toxicity and promoted glucose-stimulated insulin secretion dose-dependently. Treatment of EGCG significantly enhanced the morphology of pancreatic tissues in diabetic mice, lowered the level of blood glucose and also restored pancreatic expression of insulin mRNA.

Conclusion: The results of this study indicate that an antioxidant and anti-inflammatory agent, EGCG possesses potential benefits as a drug for treating autoimmune and inflammatory disorders.

Keywords: Epigallocatechin gallate, Type 1 diabetes, Pancreatic beta cells, T cells

In Vitro Immunomodulatory Effects of Carvacrol and Thymol on Splenocyte Cytokine Profiles and Macrophage Metabolic Polarization

Fatemeh Mashhadi[1], Majid Ghorbani Nohooji[2], Roya Yaraee[1]

1. Department of Immunology, School of Medicine, University of Shahed, Tehran, Iran.
2. Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran.

Background: Carvacrol and Thymol are two major constituents of essential oil in aromatic plants and are well known for their numerous therapeutic properties. These compounds have anti-oxidant, anticancer, diabetes prevention, cardioprotective, and antimicrobial properties. The objective of this study was to investigate the immunomodulatory effects of these components on Macrophage and T-helper (TH) cell responses.

Methods: Carvacrol and Thymol components were obtained and diluted with RPMI to a concentration of 0.001%. The splenocytes and peritoneal macrophages were isolated from BALB/c mice. After mitogen stimulation (Con-A and PMA), cells were treated with non-cytotoxic concentrations of Carvacrol and Thymol (0.001%) for 24 h. MTT assay to evaluate the cell activity was measured. The products of enzymatic pathways iNOS or arginase of macrophages were examined using NO and urea assays respectively. The release of cytokines IFN-γ, IL-4, and IL-10 in supernatants of splenocytes were measured by ELISA.

Results: We revealed that Carvacrol resulted in a significant increase of 47.6 % in macrophage proliferation ($p=0.03$). Among the compounds, only Thymol diminished the ratio M1/M2 up to about 97% of control levels ($p=0.046$). The amount of IFN-γ produced by lymphocytes treated with Carvacrol and Thymol strongly enhanced compared with non-treated cells ($p=0.000$). Significant improvements in IFN-γ/IL-4 ratio (2-fold by Carvacrol and 3.6-fold by Thymol) were seen as a result of the upregulation of Th1 markers (IFN-γ) ($p<0.01$). IL-10 levels were increased in the presence of Thymol ($P=0.024$) but both compounds also led to an improvement IFN-γ /IL-10 ratio (3.1-fold by Carvacrol and 4.8-fold by Thymol) ($p<0.01$).

Conclusion: These data showed the Immunomodulatory effects of Thymol and Carvacrol on the macrophage, as well as T-cell responses. Thymol compared to Carvacrol has anti-inflammatory effects by increasing the production of IL-10 as a primary marker of Treg and stimulating M2-like macrophage polarization. We showed in this comparative study that Thymol was more effective than Carvacrol in moderately directing the immune response to an anti-inflammatory response. However, the detailed mechanisms remain to be investigated.

Keywords: Immunomodulatory, Carvacrol, Thymol, M1/M2 Polarization, IFN-γ/IL-4, IFN-γ /IL-10

Insilico investigating the effects of Origanum vulgare flavonoids on the arginine dihydrolase pathway of Giardia lamblia

Milad Zare[1], Amir Abbas Barzegari[1], Sepideh Parvizpour[2]

1. University of Maragheh, Maragheh, Iran.
2. Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Giardia lamblia is the most common intestinal protozoan pathogen in human. This microorganism has a widespread distribution in the world and causes giardiasis. All chemical drugs that are available for the treatment of this infection have side effects. On the other hand, medicinal plants have shown promising effects in treating different kinds of parasitic diseases, including giardiasis. Origanum vulgare is a plant that has shown positive effects in the treatment of giardiasis in animal studies and in vitro. The purpose of this investigation was to examine how the flavonoids from the plant impact the key Giardia pathway.

Methods: The Giardia parasite depends heavily on the arginine dihydrolase pathway to produce ATP, which is essential for its survival. This metabolic process enables the parasite to generate up to seven to eight times more energy from arginine than from glucose, making it the most critical pathway for Giardia's survival. To study this pathway, we performed pairwise molecular docking between various flavonoids and the virulence factors. Afterward, they assessed the characteristics of each flavonoid, such as toxicity, absorption, distribution, metabolism, and excretion, using the Swiss-ADME Webserver.

Results: According to the findings of the molecular docking studies, three flavonoids (luteolin, 7-glucuronide, Naringin and Rutin) extracted from Origanum vulgare plant exhibited superior performance and are worth exploring for drug design purposes. It is worth noting that none of these three flavonoids demonstrated notable toxicity.

Conclusion: This study suggests that the flavonoids luteolin, 7-glucuronide, Naringin and Rutin extracted from the Origanum vulgare plant could be potential candidates for drug design against Giardia lamblia. These flavonoids showed superior performance in inhibiting the critical virulence factors of the parasite without showing any significant toxicity. Further studies are necessary to evaluate their efficacy and safety in vivo and their potential as alternative therapies for giardiasis.

Keywords: In silico, Giardia lamblia, Origanum vulgare, Flavonoid, Giardiasis

Investigating the anti-inflammatory effect of chamomile on peripheral blood leukocytes of patients with covid-19

Rasoul Rashidi[1], Tooba Ghazanfari[1]

1. Immunoregulation Research Center, Shahed University, Tehran, Iran

Background: Numerous studies have shown the anti-inflammatory, antimicrobial, and wound healing effects of chamomile (Matricaria chamomilla). Inflammation is a key characteristic of COVID-19. In this study, the effect of ethanolic extract of chamomile on the secretion of IL-6 and TNF-α as the markers of inflammation in cultured leukocytes of patients with COVID-19 was investigated.

Methods: peripheral blood mononuclear cells (PBMC) of three patients with COVID-19 were treated with doses of 200, 300, 400 and 500 micrograms per milliliter of ethanolic extract, doses of 0.01, 0.1 and 1 µg/ml of prednisolone (a common anti-inflammatory drug, as a positive control) and RPMI (as a negative control). After 24 hours, the viability of PBMCs and the concentration of TNF-α and IL-6 were measured by MTT and ELISA methods, respectively.

Results: Only high doses of extract chamomile (400 and 500 µg/ml) significant decreased the viability of PBMCs compared with the control; while all doses of prednisolone did not show any difference compared with the control group. All doses of ethanol extract significantly declined the concentration of IL-6 and TNF-α in compared the control group. All doses of prednisolone significantly reduced the amount of TNF-α and IL-6 cytokines. By comparing the percentage of survival and the decrease in the concentration of IL-6 and TNF-α, it was found that the doses of 200, 300 and 400 micrograms/ml of chamomile ethanol extract have a more effective anti-inflammatory effect than the cytotoxicity.

Conclusion: The results of this research suggest that the use of proper doses of chamomile extract may be regulate inflammatory consequences in COVID-19 patients.

Keywords: Chamomile Matricaria, COVID-19, inflammation, TNF-α, IL-6

Investigating the Effects of Oleuropein on Reducing the Inflammation in Rheumatoid Arthritis Patients

Kimiya Rashidan[1], Reza Jafari[2], Motahareh Dargahi[3], Niloofar Taheri[4], Fatemeh Toufan[5]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. School of Allied Medical Sciences, Shahroud University of Medical Sciences, Shahroud, Iran.
3. School of Medicine, Shahroud University of Medical Sciences. Shahroud, Iran.

Background: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory arthropathy accompanied by comorbidities and articular destruction. Although RA treatment has evolved significantly over time, many patients still do not experience prolonged remission. In spite of the numerous theories put forth, the cause of RA is still unknown. It has been established that RA is linked to immune system dysregulation and ongoing inflammation. Hence, the goal of therapy is usually to regulate inflammation. By affecting T cell subsets, TNF-alpha inhibition treatment influences the systemic immune response in RA. In addition, type 2 T cells release IL-4, a cytokine that can suppress proinflammatory reactions. Moreover, Oleuropein, a compound of olive oil, has been proven to have therapeutic benefits in different complications. Therefore, we aimed to investigate this natural product's effects on reducing RA inflammation.

Methods: 5ml blood samples were collected from 40 patients struggling with RA. Then, peripheral blood mononuclear cells (PBMCs) were isolated from each sample by Ficoll and were divided into two groups, including treatment and non-treatment. After that, all samples were added to tissue culture plates, and Oleuropein and phytohemagglutinin (PHA) were added to treatment group cells. Finally, after 72 hours, both groups TNF-α and IL-4 levels were evaluated using immunoassay supernatants. Results were analyzed by t-test and ANOVA in SPSS19.

Results: Our data showed a significant reduction in TNF-α levels was observed in PBMCs treated with Oleuropein compared to non-treated cells. This reduction peaked in the Oleuropein concentration of 100 mg/ml *($p<0.0001$).* In addition, this oil significantly increased IL-4 levels in a dose-dependent manner ($p<0.0001$).

Conclusion: Our results showed that Oleuropein has anti-inflammatory effects, as it contributes to decreasing TNF-α as well as increasing IL-4 levels. Therefore, Oleuropein could be used as a synthetic drug alternative or a supplement in the therapeutic regimen of RA patients.

Keywords: Rheumatoid Arthritis, Oleuropein, TNF-α, IL-4

Liposomal rosemary develops antitumor properties in colorectal cancer

Rouhollah Hemmati Bushehri[1], MahmoudReza Jaafari[2], Ghasem Mosayebi[3], Ali Ghazavi[4], AminReza Nikpoor[5], Ali Ganji[6]

1. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
2. Nanotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran, Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran
4. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran, Traditional and Complementary Medicine Research Center (TCMRC), Arak University of Medical Sciences, Arak, Iran
5. Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
6. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran, Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran

Background: Rosemary has antitumor properties; however, low water solubility and impaired bioavailability are limiting issues in using rosemary extract. Liposomes are synthetic vesicles with amphipathic properties, allowing the delivery of various hydrophobic and hydrophilic drugs. Features such as low toxicity, permeability, adequate bioavailability, and lack of immunogenicity and toxicity have led to the widespread use of liposomes in drug delivery systems. So, the aim of this study was to prepare liposomes (HSPC/Chol/mPEG2000-DSPE) containing rosemary alcoholic extract (LipRos) and evaluated its antitumor properties in a mouse model of colorectal cancer.

Methods: LipRos were prepared and characterized. The colorectal cancer was induced in Balb/c mice by subcutaneous injection of C26 cells, tumor size and volume was monitored continuously. The MTT assay was performed to evaluate cytotoxicity, and liver and kidney function tests were performed to evaluate its safety. Expression of apoptosis-related genes; Bax and Bcl-2, and expression of cytokines; tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), and Interferon gamma (IFN-γ) were investigated by real-time PCR. Flow cytometry assay were done to evaluate cytotoxic T lymphocytes (CTLs) and regulatory T lymphocytes (Treg cells) count in spleen and tumor tissue.

Results: The results showed that the size of liposomal formulations was 113.4 nm and their encapsulation efficiencies was 85%, The MTT assay showed insignificant cytotoxicity of LipRos on mouse splenocytes *($P>0.05$)*, and tumor size was significantly reduced in LipRos group compared to the control *($P<0.001$)*. In addition, LipRos significantly decreased Bcl-2 gene expression ($P<0.01$), increased Bax and IFN-γ gene expression ($P<0.05$), and finally increased infiltration of CTLs in tumor tissue ($P<0.05$), without significant change of TNF-α and TGF-β ($P>0.05$).

Conclusions: This study showed that PEGylated liposomes containing rosemary extract have an antitumor effect on C26 colorectal cancer cells by various mechanisms, that could be used in future studies.

Keywords: Nanoliposomes, Rosemary, Colorectal cancer, Apoptosis, Cytokine

Melatonin as an Adjuvant Treatment against COVID-19: A Systematic Review

Arezoo Faridzadeh[1], Mahmoud Mahmoudi[2]

1. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Immunology and Allergy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Since November 2019, the world has struggled with the rapidly spreading Coronavirus disease 2019 (COVID-19). The first vaccination distribution was started in December 2020 as a response to this serious health concern. However, even fully vaccinated people are susceptible to infection, although with less severe symptoms. Melatonin is an anti-inflammatory, antioxidant, and immunomodulatory drug with anti-viral capabilities, low cost, and few adverse effects that could be used as an adjuvant in the treatment of COVID-19. The clinical investigations on the effects of melatonin on COVID-19 patients are summarized in this systematic review.

Methods: The search of articles was carried out in PubMed/MEDLINE, Web of Science, Scopus, and Cochrane library up to January 2022.

Results: Ten articles were included in this study. It appears that melatonin can reduce the expression of some genes, including the signal transducer and activator of transcription (STAT)4, STAT6, T-box expressed in T cells (T-bet), GATA binding protein 3 (GATA3), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and caspase-1, as well as inflammatory markers, inflammatory cytokines, and gene expression (CASP1). It appears that melatonin can reduce the expression of some genes, including the caspase-1 (CASP1), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), GATA binding protein 3 (GATA3), T-box expressed in T cells (T-bet), the signal transducer and activator of transcription (STAT)6, STAT4, as well as inflammatory markers, inflammatory cytokines. Melatonin also seems to hasten healing and reduce several clinical symptoms and signs. Administering melatonin in severe cases reduces sepsis, thrombosis, and mortality rate.

Conclusion: This systematic review shows the possible role of melatonin as an adjuvant in the treatment of COVID-19 after about two weeks of consumption. Nevertheless, more high-quality randomized clinical trials are required.

Keywords: Melatonin, COVID-19, Systematic review.

Nano-Herbal anti-cancer agents for cancer therapy; systematic review

Soheil Sadr[1], Mehdi Soroushianfar[1], Negar Niyazmand[2], Hassan Borji[3]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of English Language and Literature, Faculty of Letters and Humanities, Ferdowsi University of Mashhad, Iran
3. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: There is no doubt that cancer is one of the leading causes of death in the world today. As of right now, there is still no effective way of curing this disease, and it has remained a major challenge for the current chemotherapy treatments. An overview of the most recent research on phytosome complexes and cancer therapy has been presented in this study. This study summarizes the strategies for synthesizing these complexes and the mechanism by which they move through phytosomes.

Methods: Eleven databases (Embase, Scopus, Google Scholar, PubMed, Cochrane Library, Magiran, UpToDate, SID, Medline, Elsevier, and Lilacs) were searched for published articles on the treatment of cancers with nano-herbals from January 2002 to January 2023. Seventeen affiliated articles with complete abstracts were included in this study. All data were extracted from interrelated papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Silibinin is a natural component extracted from silymarin and has been observed to have anti-cancer activities by decreasing N-nitrosodiethylamine in hepatocellular carcinomas. Sinigrin has healing effects on normal human keratinocytes cells (HaCaT) and concurrently investigated its phytosomal formulation anti-cancer effects on A-375 melanoma cells. Mitomycin C (MMC), a natural compound containing a carbamoyl chain and an aziridine ring, has shown potent antitumor activities; thus, it is utilized in a variety of cancers. T. arjuna (TA), a member of the Combretaceae family, the bark is rich in flavonoids and has antimutagenic anti-cancer activities. Luteolin (Lut), a natural component, has been found to have anti-cancer activities by acting on multiple molecular targets to kill cancer cells due to its potency to inhibit selectively the Nrf2 signaling pathway and sensitize non-small lung cancer cell lines.

Conclusion: Evidence suggests that phytosomal formulations of phytoconstituents may enhance their anticancer properties.

Keywords: Cancer, Phytosome, Nanotechnology, Systematic review

Naproxen as an Adjunctive Therapy in Hospitalized Patients with COVID- 19 infection: A randomized, double-blind, placebo-controlled, clinical trial

Masoomeh Asadi[1], Sara Sayar[1], Esmat Radmanesh[1], Sina Naghshi[2], Sajedeh Mousaviasl[1], Saeed Saeed[1], Mona Ebrahimzadeh[1], Asma Mohammadi[1], Samaneh Abbasi[1], Sara Mobarak[10], Saeid Bitaraf[3], Fatemeh Zardehmehri[1], Ali Cheldavi[1], Elaheh karimzadeh[4]

1. Abadan University of Medical Sciences, Abadan, Iran.
2. Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Epidemiology, Tehran University of Medical Sciences, Tehran, Iran
4. Student Research Committee, Abadan University of Medical Sciences, Abadan, Iran

Background: Naproxen is a nonselective cyclooxygenase (COX) inhibitor, which reduces the production of inflammatory mediators such as prostaglandins. The current study was done to examine the efficacy of naproxen in the management of patients with COVID-19 infection.

Methods: This study was a randomized, double-blind, placebo-controlled, clinical trial that was done in Abadan, Iran, in 2020. Patients were randomly assigned to receive either naproxen (two capsules per day each containing 500 mg naproxen sodium) or placebo (containing starch) for five days along with the routine treatment that was nationally recommended for COVID-19 infection. Clinical symptoms of COVID-19 infection, the time to clinical improvement, blood pressure, laboratory parameters, and death due to COVID-19 infection were considered as the outcome variables in the present study. The ethics committee of the Abadan School of Medical Sciences approved the study (with code of IR.ABADANUMS.REC.1398.115). Moreover, this study was registered in the Iranian Registry of Clinical Trials (www.irct.ir) on 2020-03-30 with the code number IRCT20200324046850N3.

Results: Treatment with naproxen improved cough and shortness of breath in COVID-19 patients; such that, compared with placebo, naproxen intake was associated with 2.90 (95% CI: 1.10e7.66) and 2.82 (95% CI: 1.05e7.55) times more improvement in cough and shortness of breath, respectively. In addition, naproxen administration resulted in a significant increase in mean corpuscular volume (MCV) and had a preventive effect on the reduction of systolic blood pressure in COVID-19 patients.

Conclusion: Treatment with naproxen can improve cough and shortness of breath in COVID-19-infected patients. Further studies are required to confirm our findings.

Keywords: COVID-19, SARS-CoV2, prostaglandin, Naproxen

Response of T lymphocytes in mice challenged with ovalbumin and treated with sumac

Elham Naji Zavareh[1], Seyyed Meysam Abtahi Froushani[1]

1. Urmia University of Medical nSciences, Urmia, Iran.

Background: Sumac (Rhus coriaria) is a commonly used spice, condiment, and flavoring agent. This plant is rich in various classes of phytochemicals including tannins, flavonoids, and polyphenolic compounds. This study was done to evaluate the effects of the hydroalcoholic extract of sumac on in vivo and ex vivo T helper (Th) lymphocyte responses and their subsets in mice immunized with ovalbumin (OVA).

Methods: Male NMRI mice were randomly divided into three groups (n=5) as follows: mice treated with PBS, OVA-immunized mice, OVA-immunized mice daily treated with hydroalcoholic extract of sumac (40 mg/kg) from two days prior to immunization to 28 days after immunization. OVA (at 2 mg/ml in PBS) was emulsified in an equal volume of Complete Freund's adjuvant (CFA), and then 0.1 ml of the emulsion was injected SC into the animals' shaved backs. Also, the mice were boosted ten days later with the same concentration of OVA as in the initial challenge in incomplete Freund's adjuvant (IFA).

Results: In vivo results revealed that OVA-challenged rats treated with hydroalcoholic extract of sumac had decreased delayed-type hypersensitivity comparable to control mice. The splenocyte proliferation index was significantly decreased in sumac-received rats compared to untreated rats. In the splenocyte cultures, sumac-treated mice showed a remarkable decrease in T-bet and RORγt expression and conversely demonstrated a significant increase in the expression of FOXP3. However, receiving sumac did not lead to changes in the expression of factor A in mice challenged with ovalbumin.

Conclusion: As the hydroalcoholic extract of sumac inhibited antigen-specific lymphocyte proliferation and was incapable of altering the level of Th2 responses, this indicated its potential to act as an immunomodulator instated of immunosuppressive properties.

Keywords: Sumac; Immunization; Immunomodulation; Immunosuppression.

Salvurmin A and Salvurmin B, Two Ursane Triterpenoids of Salvia Urmiensis Induce Apoptosis and Cell Cycle Arrest in Human Lung Carcinoma Cells

Shima Hashemi[1], Nasrollah Erfani[2]

1. Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

Background: Cancer is still the second leading cause of death in human society. Ursane triterpenoids which are a main group of natural products, could be considered as novel multi-target therapeutic anti-cancer agents. Salvurmin A and B are novel cytotoxic ursane triterpenoids isolated from the aerial parts of Salvia urmiensis, an endemic plant species of Iran.

Methods: In this study, we assessed cytotoxicity of these compounds against two human cancer cell lines: human colon adenocarcinoma cells (HT29), and human alveolar lung carcinoma cells (A549) and investigated its antiproliferative effects via apoptosis and cell cycle arrest.

Results: Salvurmin A and B showed the most cytotoxic effect on A549 cells compared to HT29 cells. IC50 values for Salvurmin A and B against A549 cells were 35.6 ± 1.5 and 19.2 ± 0.8 µM, respectively. Based on annexin V staining, both of these compounds significantly induced apoptosis in A549 cells and the apoptotic induction was dose-dependent. Moreover, these two compounds dramatically increased cell accumulation in G2/M and decreased the number of cells in G0/G1 phases in A549 cells in a dose dependent manner.

Conclusions: Based on the results Salvurmin B can be considered as potential candidate for further studies against human lung carcinoma.

Keywords: Salvia urmiensis, Salvurmin A and B, Ursane triterpenoids, Apoptosis and Cell cycle arrest.

The anti-cancer effect of pomegranate extract on MCF 7 cell line

Nafiseh Paysokhan[1], Arefeh Majidi khameneh[1], Zahra Matluobi[1]

1. Cellular and Molecular Research Center Affiliated to Sabzevar University of Medical science, Sabzevar, Iran.

Background: In these modern days with the development of science and technology, scientists tried to find the best and most effective way to treat cancer. Using herbal extract beside chemicals is a way to reduce the side effect of chemotherapy and at the same time increase the effect. One of the candidate extracts is pomegranate extract. Pomegranate extract contains ellagitannin, which is a precursor of ellagic acid. Ellagitannin has strong apoptotic and antioxidant properties. Unlike ellagic acid, ellagitannin is soluble. In this study, we decided to test pomegranate extract that contains ellagitannin. MCF 7 cells were used for this purpose.

Methods: After cultivation of MCF 7 cell line in 24-well plates, they were seeded. Complete culture medium containing 10% FBS (RPMI) was used and placed in an incubator with humidity of 90%. After 24 hours, pre-prepared pomegranate extract that was sterilized by filtration was added to each well in specific proportions. In order to check on normal cells, it was used on the cells of fresh blood lymphocytes. After 2 hours, the toxicity level was measured by MTT assay.

Results: MTT assay shows that the MCF 7 cancer cells were killed to a good extent and the lymphocyte cells survived in about 90%. The highest death rate of MCF 7 cells in 500 µl was 76%. In 250 µl of pomegranate extract, the rate of cell death was equal to 45%, and in 100 µl, it was equal to 24%.

Conclusion: Pomegranate extract can be used as a complementary drug besides chemotherapy, although this research can be tested on other cancer cells as well.

Keywords: pomegranate extract, MCF 7, cancer, ellagitannin

The effect of three medicinal herbs of traditional medicine on immunological changes in type 2 diabetes patients

Nazanin Shahnoruzi[1], Mehdi Salehi[2], Ali Ganjy[3], Ghasem Mosayebi[3], Ali Ghazavi[1]

1. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
2. Traditional and Complementary Medicine Research Center (TCMRC), Arak University of Medical Sciences, Arak, Iran
3. Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran

Background: Type 2 diabetes mellitus (T2DM) is a heterogeneous disease, and the immune system is involved in its pathogenesis by causing inflammation and oxidative stress. The aim of this study was to investigate the immunomodulatory effects of an herbal supplement containing Fenugreek, Nigella sativa, and Sumac in patients with T2DM.

Methods: Fifty patients with T2DM were randomly divided into two groups: the intervention (treatment with an herbal supplement containing Fenugreek, Nigella sativa, and Sumac) and placebo. The patients of the intervention group received three capsules daily for six weeks. The percentage of Treg cells by flow cytometry, the gene expression of IL-17, IFN-γ, TNF-α, and TGF-β by real-time PCR, serum antioxidant capacity by FRAP, and serum nitric oxide level by the Griess technique was determined.

Results: The results of this study showed that T2DM patients had a higher gene expression of TGF-β *(p=0.045)* while decreased expressions of Treg cells *(p=0.001)* and Treg cells markers (CD25 *(p=0.049)* and Foxp3 *(p=0.017*)) compared to healthy controls. The administration of the herbal supplement for six weeks could not correct immunological changes in patients with T2DM.

Conclusion: The present study showed that the daily administration of herbal capsules containing 1500 mg of Fenugreek, Nigella sativa, and Sumac for six weeks had no remarkable effects on the immunomodulatory markers measured. It is suggested to evaluate the immunomodulatory effects of these plants at a higher dose and over a longer period of time.

Keywords: 'Type 2 diabetes, Fenugreek, Nigella sativa, Sumac

The effects of Apigenin on the inhibition of inflammatory responses and oxidative stress in the lung injury: A systematic review and meta-analysis

Ali Rahimi[1]

1. Iran University of Medical Sciences, Tehran, Iran.

Background: Apigenin is a member of the flavonoid family that can regulate various biological processes, which is characterized as a treatment of different inflammatory disorders and pathological problems associated with oxidative stress (OS). Recent research has focused on apigenin immunomodulatory properties as a potential treatment for different types of lung injuries. This meta-analysis was designed to determine the impact of apigenin treatment on inflammatory markers and OS parameters in animal models of lung injuries.

Methods: The comprehensive literature search was conducted using electronic databases such as Google Scholar, PubMed, Web of Science, Scopus, and Embase up to August 2021. To assess apigenin's effect on inflammatory mediators and OS biomarkers in lung injury animal models, we used the I2 statistic to determine the heterogeneity. We then pooled data as standardized mean difference (SMD) with a 95% confidence interval (CI).

Results: Our meta-analysis of the pooled data for inflammatory biomarkers demonstrated that the apigenin administration significantly decreased the NF-κB expression (SMD − 1.60, 95% CI [− 2.93 to − 0.26]; I2 = 89.0%, $p < 0.001$), IL-1β (SMD − 4.30, 95% CI [− 6.24 to − 2.37]; I2 = 67.3%, $p = 0.047$), IL-6 (SMD − 4.10, 95% CI [− 5.04 to − 3.16]; I2 = 72.6%, $p < 0.001$) and TNF-α (SMD − 3.74, 95% CI [− 4.67 to − 2.82]; I2 = 84.1%, $p < 0.001$). This study also indicated the efficacy of apigenin in increasing the level of CAT (SMD 4.56, 95% CI [3.57 to 5.55]; I2 = 15.3%, $p = 3.15$), GSH (SMD 5.12, 95% CI [3.53 to 6.70]; I2 = 77.6%, $p < 0.001$), and SOD (SMD 3.45, 95% CI [2.50 to 4.40]; I2 = 79.2%, $p < 0.001$), and decreasing the level of MDA (SMD − 3.87, 95% CI [− 5.25 to − 2.49]; I2 = 80.3%, $p < 0.001$) and MPO (SMD − 4.02, 95% CI [− 5.64 to − 2.40]; I2 = 88.9%, $p < 0.001$), TGF− β (SMD − 3.81, 95% CI [− 4.91 to − 2.70]; I2 = 73.4%, $p=0.001$) and W/D level (SMD − 3.22, 95% CI [− 4.47 to − 1.97]; I2 = 82.1%, $p< 0.001$) than control groups.

Conclusion: Overall, our findings showed the immunomodulatory potential of apigenin as an alternative treatment for the suppression of inflammatory responses and OS in different types of lung injury diseases. Nevertheless, due to the paucity of clinical studies, reliable preclinical models, and clinical settings, evaluating the influence of apigenin on lung injury is required in the future. Before conducting large-scale clinical trials, detailed human pharmacokinetic studies are also needed to establish dosage ranges and determine the initial safety and tolerability of apigenin.

Keywords: Apigenin, Acute lung injury (ALI), Lung fibrosis, Inflammation

The effects piperine on Experimental autoimmune uveitis

Alireza Ghavami[1], Seyyed Meysam Abtahi Foroushani[2], Ali Asghar Tehrani[3]

1. PhD student of Immunology Department, Urmia University, Urmia, Iran.
2. Associate Professor and Director of Immunology Department, Urmia University, Urmia, Iran.
3. Associate Professor of Pathobiology Department, Urmia University, Urmia, Iran.

Background: Uveitis is a common eye disease that threatens vision. It can be due to both infectious and non-infectious causes, caused by damage to the eye, viral or bacterial infection, and autoimmune uveitis, especially if untreated, can lead to visual impairment and blindness. 5 to 20% of blindness in countries developed and includes 25% in developing countries. Although the anti-inflammatory and immunomodulatory effects of piperine have been mentioned in past studies, but there is no evidence about the role of piperine in ameliorating experimental model of autoimmune uveitis (EAU). Piperine is one of the most common spices around the world, it is the main active ingredient of black pepper. In traditional medicine, black pepper is used as an analgesic and anti-inflammatory.

Methods: EAU induced in Lewis rats by immunization with interphotoreceptor retinoid-binding peptide (IRBP) emulsified in complete Freund adjuvant. Treatment groups daily received piperine (20, 40 and 80 mg/kg-p.o.) or prednisolone (2 mg/kg-p.o.) from day 8 post immunization when the animals developed the first signs of uveitis and continued throughout the investigation until the day18 when rats were sacrificed.

Results: Clinical and histopathological findings represented severe intraocular inflammation and significant weight loss in the immunized rat. All treatment protocols were successful in reducing clinical symptoms and leukocyte infiltration into the retina. Piperine at a dose of 80 mg/kg was as effective as prednisolone in reducing the mean clinical score, histopathological changes and Indoleamine 2, 3-dioxygenase activity in the eyes. In terms of improving weight gain, piperine worked better than prednisolone. In terms of improving weight gain, 80 mg/kg piperine worked better than prednisolone. Nevertheless, prednisolone performed better in reducing nitric oxide levels in the eyes of EAU rats than other groups. The mRNA expression of IL-10 and TGF-β in the eyes of EAU rats received 80 mg/kg piperine was significantly mounted more favorable than other treatment groups. The ratios of T-bet/GATA-3, RORγc/T-bet, T-bet/Foxp3, RoRγc/Foxp3, T-bet/GATA-3, and RORγc/GATA-3 expression represented a further decrease in the EAU rats treated with 80 mg/kg piperine or prednisolone compared to other groups. Ex vivo stimulation of splenocytes from EAU rats treated with piperine or prednisolone showed a significant IRBP-specific proliferation compared to splenocytes from un-treated animals.

Conclusion: Oral administration of piperine shows beneficial effects in alleviating the EAU and pierine may be a potential clinical application in uveitis. Key words: autoimmunity, experimental uveitis, piperine, prednisolone.

Keywords: Key words: autoimmunity, experimental uveitis, piperine, prednisolone

The gold nanoparticles synthesized using the aqueous extract of Allium saralicum and zinc effectively prevent induced gastroduodenal ulcer

Mohammad Mehdi Zangeneh[1], Mahtab Pourkamalzadeh[2], Navid Etemadi[3], Akram Zangeneh[4]

1. Biotechnology and Medicinal Plants Research Center Ilam University of Medical Sciences, Ilam, Iran.
2. Department of Microbiology, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.
3. Veterinary Graduate, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
4. Biotechnology and Medicinal Plants Research Center Ilam University of Medical Sciences, Ilam, Iran.

Background: Because many people in the world suffer from gastroduodenal ulcers, therefore, studying the therapeutic strategies of these ulcers are research priorities in any country. The aim of the new study was to survey the preventive property of gold and zinc nanoparticles Allium saralicum on ethanol-induced gastroduodenal ulcers in rats.

Methods: In this study, 42 rats were used. The rodents were randomly divided into six subgroups, including negative healthy control receiving distilled water, untreated negative control receiving distilled water, positive control receiving omeprazole 60 mg/kg, one group receiving the aqueous extract of Allium saralicum at 200 mg/kg concentrations , a group receiving the of gold nanoparticles of aqueous extract Allium saralicum at 5 mg/kg concentrations and a group receiving the of zinc nanoparticles of aqueous extract Allium saralicum at 5 mg/kg concentrations. After 14 days, gastroduodenal ulcers were caused by ethanol. Four hours after oral administration of ethanol, the rats were dissected and blood, stomach, and duodenum samples of them collected for hematological, biochemical, Immunology, and gross parameters analysis. The data were analyzed using one-way ANOVA followed by Duncan post hoc test.

Results: The gold nanoparticles of Allium saralicum aqueous extract could significantly ($p \leq 0.05$) decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the Other groups. Also, the gold nanoparticles of aqueous extract prevented significantly ($p \leq 0.05$) small, medium and large gastroduodenal ulcers as compared to the other groups. It also further reduces NO, MPO and MDA compared to other groups.

Conclusion: It seems that the gold nanoparticles of Allium saralicum aqueous extract can prevent gastroduodenal ulcers in rats without any side effect.

Keywords: gold nanoparticles (AuNPs) of Allium saralicum aqueous extract; gastroduodenal protective; ethanol.

The role of synthesized Falcaria vulgaris nanoparticles in the management of non-alcoholic fatty liver disease

Maryam Almasi[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[2], Seyed Meysam Abtahi Foroushani[3], Hori Ghaneialvar[1], Mohammad Abbaszadeh[4]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
2. Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran
3. Department of Microbiology, Veterinary Faculty, Urmia University, Urmia, Iran.
6. Quality Control Laboratory, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Background: Fatty liver disease is the accumulation of fat in liver cells and in the absence of early diagnosis and proper treatment, it can become an advanced disease called cirrhosis. In the traditional medicine of western Iran, the Falcaria vulgaris plant with the local name of Paghazeh is used to treat stomach ulcers and accelerate the healing of skin wounds. We decided to help improve fatty liver disease by using Paghaze nanoparticles.

Methods: At first, we select 7 healthy rats as negative control and we divide 28 rats with high-fat diet into 4 groups during two months of treatment and gavage was given: untreated negative control (Physiological Serum), Falcaria vulgaris aqueous extract (160 mg/kg), Falcaria vulgaris silver nanoparticle group (0/4 mg/kg), Falcaria vulgaris zink nanoparticle group (0/4 mg/kg). After euthanasia, their blood and liver samples were taken. The quantitative data were evaluated by SPSS-26 software using one-way ANOVA followed by Duncan test. To analyze the histological data, the Kruskal-Wallis test was run.

Results: Zink and silver nanoparticles of Falcaria vulgaris could significantly ($p \leq 0.05$) increase the concentrations of HDL, total protein, albumin, SOD, CAT, and GPx and decrease the raised weights of body and liver and the concentrations of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, glucose, and GR as compared to the other groups. It also further reduces NO, MPO and MDA .Also zink and silver nanoparticles of Falcaria vulgaris reduced the degree of hepatic steatosis as compared to the other groups.

Conclusion: It seems that nanoparticles of Falcaria vulgaris can be effective in improving fatty liver by reducing liver enzymes and lipid profile.

Keywords: nanoparticles, fatty liver, Falcaria vulgaris, liver enzymes

The silver and zinc nanoparticles synthesized using the aqueous extract of Allium Saralicum R.M. Fritsch effectively prevent induced ulcerative colitis

Somayeh Ahmadiafshar[1], Mohammad Mehdi zangeneh[2], Huri Ghaneialvar[2], Neda Ahmadiafshar[3], Akram zangeneh[1]

1. Urmia University of Medical Sciences, Urmia, Iran.
2. Ilam University of Medical Sciences, Ilam, Iran.
3. Tehran University of Medical Sciences, Tehran, Iran.

Background: The use of medicinal plants is the oldest method for treating various diseases. Many plants have been recommended for the treatment of ulcerative colitis. The aim of this study was to investigate the antioxidant activities and protective effect of silver and zinc nanoparticles against ulcerative colitis in rats.

Methods: In this study, 40 adult male rats were divided into 5 groups, randomly: Healthy control group, Patient control group, Patient group receiving Prednisolone (60 mg/kg), Patient group receiving Allium Saralicum R.M. Fritsch aqueous extract (200mg/kg), Patient group receiving silver and zinc nanoparticles of Allium Saralicum R.M. Fritsch aqueous extract (0/5 mg/kg).To induce ulcerative colitis, acetic acid was injected intra rectally. After confirming the induction of the disease, treatment period was started in rats. After ten days, plasma and serum of rats were sent for hematological and biochemical tests and intestines of rats for histopathology.

Results: The results showed that the level of AST, ALT, ALP, GGT, cholesterol, LDL, triglyceride, Creatinine, HDL, total protein, WBC, hemoglobin, RBC, PCV, MCV, MCH and MPO, MDA,NO and also necrosis, erythrocyte congestion and accumulation of inflammatory cells in the intestinal tract were significantly reduced in the group receiving nanoparticles compared to other groups. On the other hand, macroscopic examination of tissues showed a significant decrease in the level of all three types of small, large and medium wounds in this group compared to other groups.

Conclusion: This study showed that the use of silver and zinc nanoparticles containing Allium Saralicum R.M. Fritsch extracts has significant antioxidant and protective effects compared to the use of this plant alone or the use of prednisolone in the treatment of ulcerative colitis in rats.

Keywords: silver and zinc nanoparticles, Allium Saralicum, ulcerative colitis, Rats

The silver nanoparticles synthesized using the aqueous extract of Artemisia (Mugworts) effectively prevent induced gastroduodenal ulcer

Mohammad Mehdi Zangeneh[1], Neda Ahmadiafshar[2], Naser Abbasi[2], Samira Zand[1], Akram Zangeneh[1], Somayeh Ahmadiafshar[1]

1. Ilam University of Medical Sciences, Ilam, Iran.
2. Tehran University of Medical Sciences, Tehran, Iran.

Background: Peptic ulcer is a common disorder of gastrointestinal system. Therefore, studying the therapeutic strategies of these ulcers are of great importance. The aim of the new study was to survey the preventive property of silver nanoparticles of Artemisia (Mugworts) on ethanol-induced gastroduodenal ulcers in rats.

Methods: In this study, 35 adult male rats were divided into 5 groups, randomly: negative healthy control receiving distilled water, untreated negative control receiving distilled water, positive control receiving omeprazole 60 mg/kg, one group receiving the aqueous extract of Artemisia (Mugworts) at 200 mg/kg concentrations, and a group receiving the of silver nanoparticles of aqueous extract Artemisia (Mugworts) at 5 mg/kg concentrations. After 14 days, gastroduodenal ulcers were caused by ethanol. Four hours after oral administration of ethanol, the rats were dissected and blood, stomach, and duodenum samples of them collected for hematological, biochemical, Immunology, and gross parameters analysis.

Results: The silver nanoparticles of aqueous extract Artemisia (Mugworts) could significantly decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the Other groups. Also, the silver nanoparticles of aqueous extract prevented significantly small, medium and large gastroduodenal ulcers as compared to the other groups. It also further reduces NO, MPO and MDA compared to other groups.

Conclusion: It seems that the silver nanoparticles of aqueous extract Artemisia (Mugworts) can prevent gastroduodenal ulcers in rats without any side effect.

Keywords: silver nanoparticles, Artemisia, gastroduodenal ulcer, Rat

The therapeutic efficacy of Tranilast in combination with antiviral drugs in hospitalized COVID-19 patients: A randomized controlled trial

Ali Saeedi-Boroujeni[1], Roohangiz Nashibi[2], Ata A Ghadiri[3], Motowo Nakajima[4], Shokrollah Salmanzadeh[5], Mohammad-Reza Mahmoudian-Sani[6], Mohammad Ghasem Hanafi[7], Asaad Sharhani[8], Ali Khodadadi[9]

1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Infectious Diseases & Tropical Medicine Ward, Razi Teaching Hospital, Ahvaz Jundishapur University of Medical Science
3. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4. Department of R&D, SBI Pharma Co., Ltd., Tokyo 106-6020, Japan
5. Infectious Diseases & Tropical Medicine Ward, Razi Teaching Hospital, Ahvaz Jundishapur University of Medical Science
6. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
7. Department of Radiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
8. Department of Epidemiology and Biostatistics, School of public health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
9. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. 7. Cancer Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: To evaluate the therapeutic effects of Tranilast in combination with antiviral drugs in non-ICU-admitted hospitalized patients with COVID-19.

Methods: This study was a clinical trial performed in the infectious ward of Razi Hospital in Ahvaz. Patients were randomly assigned in a 1:1 ratio to control (30) and intervention groups (30). Patients in the control group received antiviral therapy, while patients in the intervention group received Tranilast (300 mg daily) in addition to the antiviral drugs for Seven days. The collected data, including inflammatory cytokine expression, laboratory tests, and clinical findings, was used for intragroup comparisons. The Ethics Committee approved the study of the Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1399.050). Furthermore, this study was registered in the Iranian Registry of Clinical Trials (IRCT20200419047128N1).

Results: The intervention group showed significantly lower levels of NLR (*p=0.001*), q-CRP (*p=0.002*), IL-1 (*p=0.001*), TNF *(p=0.001*), and LDH (*p=0.046*) in comparison with the control group. The effect of the intervention was significant in increasing the o2 saturation (*F=7.72, p=0.007*). Extended hospitalization (four days or above) was 36.6% in the Tranilast and 66.6% in the control group (RR = 0.58; 95% CI: 0.38–1.06, *p=0.045*). In the Trails and control groups, one and four deaths or hospitalization in ICU were observed respectively (RR = 0.31; 95% CI: 0.03–2.88, *p=0.20*).

Conclusion: Tranilast might be used as an effective and safe adjuvant therapy and enhance the antiviral therapy's efficacy for managing patients with COVID-19Keywords: COVID-19, cytokine storm, SARS-CoV-2, Tranilast.

Keywords: COVID-19, cytokine storm, SARS-CoV-2, Tranilast

Therapeutic Efficacy of Evening Primrose Oil in Patients with Rheumatoid Arthritis: A Randomized Double-Blind, Placebo-Controlled Clinical Trial

Bahareh Abd Nikfarjam[1]

1. Department of Immunology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran

Background: Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease. In recent years, new drugs with novel targets have been developed to increase the efficacy of drugs in the treatment of RA. The pharmacological therapy of RA is often symptomatic to mitigate pain and inability with analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), drugs with defined side effects and risks. Complementary medicines might decrease the signs of RA and reduce the need for these medicines. In the present study, we studied anti-inflammatory and antioxidant effects of medicinal plants Evening Primrose Oil, in patients with RA. Moreover, the effects of these herbal medicines on IL-17 production has not been investigated in RA patients.

Methods: This randomized, double-blind, controlled trial selected 60 eligible RA patients for three months, and randomly divided them into Urtica dioica, and placebo groups. Moreover, the potential effect of these herbal medicines on Disease Activity Score (DAS) 28, Total Anti-oxidant Capacity (TAC), IL-17, Rheumatoid Factor (RF), anti-cyclic citrullinated peptide antibodies (Anti-CCP), C Reactive Protein (CRP), and Erythrocyte Sedimentation Rate (ESR) before and after clinical trial were evaluated.

Results: After a three-month follow-up, the mean values of DAS28, IL-17, TAC, RF, and CRP in EPO group were significantly different from the placebo group. However, the VAS, Anti-CCP, and ESR at baseline and at the end of the study were not significantly different between the two groups. After the intervention, the within-group DAS28 in the EPO and placebo groups reduced significantly compared to the baseline.

Conclusion: Medicinal plant Evening Primrose Oil appeared to decrease the symptoms and inflammatory factors, and can improve the signs of RA. Thus, Urtica dioica has a great potential as a complementary therapy in patients with RA and other chronic inflammatory diseases.

Keywords: Rheumatoid Arthritis, Evening Primrose Oil, Clinical Trial, IL-17, TAC

Three medicinal plants of traditional medicine on the response of misfortune in the second type

Maryam Khaltabari Limaki[1], Mehdi Salehi[2], Ghasem Mosayebi[3], Ali Ganji[4], Ali Ghazavi[5]

1. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
2. Traditional and Complementary Medicine Research Center (TCMRC), Arak University of Medical Sciences, Arak, Iran
3. Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran
4. 4Infectious Diseases Research Center (IDRC), Arak University of Medical Sciences, Arak, Iran
5. Department of Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.

Background: Since in patients with type 2 diabetes (T2DM), there is an imbalance of inflammatory cells, the presence of inflammatory cytokines and defects in homeostasis are possible. The aim of this study was to investigate the immunomodulatory effects of an herbal supplement.

Methods: Fifty patients with T2DM and 50 healthy controls were included in this study, according to the inclusion criteria. Patients received 3 capsules containing supplements daily for 6 weeks. The percentage of Treg cells, T cells, and T helper cells was determined by flow cytometry. Also, the expression of CD4, CD25, and FOXP3 markers of Treg cells was examined. The gene expression of TGF-β cytokine was evaluated by real-time polymerase chain reaction (PCR).

Results: The percentage of Treg cells was significantly lower in patients with T2DM than in healthy controls. The number of T helper cells and lymphocytes decreased in T2DM patients as compared to the healthy controls. Based on the results, the percentage of T cells was higher in T2DM patients than in healthy controls. The expression of CD25 and FOXP3 markers in Treg cells were significantly reduced in T2DM patients compared to the healthy controls; however, this decrease was not significant for the CD4 marker. Conversely, the expression of TGF-β cytokine increased in patients with T2DM compared to the healthy controls. Taking herbal supplements for 6 weeks could not correct the immunological changes in T2DM patients.

Conclusion: TGF-β and CD4 + CD25 + regulatory T cells are impaired in patients with type 2 diabetes.

Keywords: Keywords: herbal supplement, regulatory T cells, transforming growth factor-beta (TGF-β), type 2 diabetes mellitus (T2DM)

Immunogenetics

A systematic review on the potential of circulatory cell free DNA in the diagnosis of Systemic lupus erythematosus patients

Ramin Yousefpour Shahrivar[1], Zahra Ahmadi Fakhr[1], Zahra Jahanbakhsh[1], Fatemeh Karami[2]

1. Department of Biology, College of Convergent Sciences and Technologies, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Medical Genetics, College of Medical Sciences and Technologies, Applied Biophotonics Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran

Background: Systemic lupus erythematosus (SLE) is one of the frequent autoimmune disorders which is characterized by production of auto-antibodies against different tissues and circulatory DNAs released from apoptotic bodies. Owing to the confusing and delayed diagnosis of SLE, demand for early biomarkers made me to assess the potential of circulatory cell free DNA (cfDNA) in the diagnosis of SLE patients through systematic review of the previously performed studies.

Methods: An advanced literature search was conducted in PubMed, Google Scholar and Embase databases according to the PRISMA guidelines. Searching was included following keywords: [("SLE" OR "Systemic Lupus Erythematosus" OR "Lupus")] AND [("Cell free DNA" OR "Cell-free mitochondrial DNA")] AND [("quantity")]. All the articles since 2016 was included in the present study.

Results: Among ten finally included studies for review, nine articles have approved that cfDNA significantly increases in SLE patients compared to healthy controls. The remained one study demonstrated that higher cfDNA quantity alternatively occurs in circulating microparticles. In contrast, Cell- free mitochondrial DNA (cf mt-DNA) was shown in eight studies to be significantly decreased in SLE patients which unlike cfDNA was negatively correlated with disease severity.

Conclusion: High cfDNA and low cf mt-DNA quantity can be potentially used as biomarkers in early diagnosis of suspicious SLE cases. Further studies are required to shed light on the genetic and epigenetic alteration of cfDNA before and in response to treatment.

Keywords: Systemic lupus erythematosus (SLE), Cell free DNA, Cell- free mitochondrial DNA, quantity

Association between histamine metabolism genes variants (HNMT and ABP1) and H1 antihistamines response in patients with chronic urticaria

Fatemeh Zamani[1]

1. Lung Diseases and Allergy Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran

Background: Previous studies have shown that some single nucleotide polymorphisms (SNPs) in histamine-metabolizing genes are linked to the severity of allergic disorders, including chronic urticaria. The current study aims to investigate the connection between histamine metabolism genes variants, including histamine N-methyl transferase (HNMT) rs11558538 and amiloride binding protein 1 (ABP1) rs1049793, and H1 antihistamines responsiveness in chronic urticaria patients.

Methods: A total of 126 people with chronic urticaria were included in this prospective study. Genomic DNA was extracted from blood samples using the DNA extraction kit. HNMT rs11558538 and ABP1 rs1049793 were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: The mean score of urticaria severity in the first week was 11.12 ± 5.034 and in the 12th week of treatment was 3.80 ± 3.476. After the first week of treatment, the mean D-dimer level and urticaria severity score were significantly higher in the CG genotype group than in the CC genotype group for the ABP1 gene (p<0.01). Although the severity of urticaria was higher in the CG genotype group, the ratio of response to treatment and reduction of urticaria severity after the 12th week of treatment in this genotype was more than twice that of the CC genotype group.

Conclusion: Our findings indicated that patients with chronic urticaria who had the heterozygous variant of ABP1 and HNMT genes (CG and CT, respectively) had a better response to treatment than patients with variant CC of any of the mentioned genes.

Keywords: Chronic urticaria, HNMT, Antihistamines, ABP1

Combination effect of killer cell immunoglobulin-like receptors (KIRs) and their cognate HLA class I ligands on susceptibility to acute myeloid leukemia in Iranian patients

Sara Mirzazadeh[1], Peyman Bemani[2], Hossein Halimi[1], Shirin Farjadian[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Background: Acute myeloid leukemia (AML) is broad and heterogeneous in genetic background and disease outcome. Killer immunoglobulin-like receptors (KIR) and Human leukocyte antigen class I (HLA-I) are two families of genes that have a significant impact on NK cell immunosurveillance function. For this reason, understanding the KIR/HLA combination effect on anti-cancer defense is necessary, but it is a formidable challenge; in fact, we tried to give a comprehensive insight into KIR/HLA combinatorial diversity role in predisposition to AML. Therefore, the objective of this study was to investigate the association of KIR/HLA-I combinations with susceptibility to AML in the Southwestern Iranian population.

Methods: PCR-SSP by some unique primers for 181 AML patients and 181 healthy controls was applied to determine the KIR and HLA gene contents.

Results: According to our results, the frequencies of KIR3DS1 (p=0.0001, OR=2.32), KIR2DS4fl (p=0.02, OR=1.53), CxT4 genotypes (p=0.04, OR=2.0), and T4 genes cluster (p=0.01, OR=1.99) were significantly higher in AML patients than the controls, while HLA-C1 (p=0.05, OR=0.61), C2 (p=0.01, OR=0.54), HLA-A Bw4 (p=0.03, OR=0.6), and HLA-A11 (p=0.04, OR=0.57) were more frequent in the controls. In addition, inhibitory (i) KIR/HLA-I combinations analysis revealed higher frequencies of KIR2DL1(+)/HLA-C2(+), KIR2DL2,3(+)/HLA-C1(+), and KIR3DL2(+)/HLA-A3,11(+) in the control group (p=0.003, OR=0.49; p=0.05, OR=0.62; and p=0.03, OR=0.62 respectively). Overall, the number of iKIR/HLA-I combinations was more in the control group. Moreover, KIR3DS1(+)/HLA-B Bw4Ile80(+) and the sum of HLA-B Bw4/A Bw4 combined with KIR3DS1 as aKIR/HLA-I combinations were more frequent among the patients than the controls (p=0.02, OR=1.99, and p=0.007, OR=1.97, respectively).

Conclusion: We postulate that iKIR/HLA-I combinations might be protective factors against AML because of possessing a beneficial effect on NK education fate. It is noteworthy that KIR/HLA-I combination studies can be applicable in donor selection for allogeneic NK cell therapy in hematological malignancies.

Keywords: "Acute myeloid leukemia", "Killer immunoglobulin-like receptors", "Human leukocyte antigen", "KIR/HLA-I combination", "Immunogenetics"

Correlation of Gene polymorphism and Serum levels of Interleukin-37 with the risk of Coronary Heart Disease

Samaneh Tabaee[1], Hassan Dana[2], Samira Tabaei[3]

1. Noncommunicable Disease Research Center, Neyshabur University of Medical Science, Neyshabur, Iran
2. Faculty of Medicine, Neyshabur University of Medical Science, Neyshabur, Iran
3. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Interleukin 37 (IL-37) has an anti-inflammatory effect involved in the pathogenesis of coronary heart disease (CHD). This study was aimed to evaluate the association of two IL-37 polymorphisms and IL-37 serum level with pathological characterization of the CHD patients and healthy controls.

Methods: In this case control study, two IL-37 polymorphisms (rs2708961, rs6717710) were genotyped in 145 patients with CHD and 136 healthy controls using amplification refractory mutation system-PCR (ARMS-PCR). In addition, serum level of IL-37 was measured in CHD and healthy controls by commercial ELISA kit. Statistical analysis was performed using SPSS software.

Results: Our results indicated a significant difference in the allelic and genotypic distribution of two IL-37 polymorphisms were found between CHD patients and healthy controls. Also, we found an increasing tendency in IL-37 serum level in patients with CHD (42.77 ± 3.36 pg/mL) in comparison to controls (46.70 ± 3.72 pg/mL, p=0.007) in comparison with heathy controls (29.96 ± 3.30 pg/mL, p=0.03).

Conclusion: These results suggest that IL-37 is associated with susceptibility to CHD and could serve as a prognostic biomarker in coronary heart patients.

Keywords: Coronary heart disease, Polymorphism, IL-37, ELISA

Dysregulated miRNAs triggering inflammatory pathways in Systemic Lupus Erythematosus

Benyamin Gheiji[1]

1. Student Research Committee, Mashhad University of Medical Science, Mashhad, Iran.

Background: Systemic Lupus Erythematosus (SLE), is a chronic autoimmune disease that affects multiple organs and is characterized by the presence of autoantibodies, inflammation, and tissue damage. As a multifactorial disease, SLE's complex etiology involves genetic, environmental, and immunological components. MicroRNAs (miRNAs) are small non-coding regulating the immune system and inflammatory responses. Dysregulation of miRNAs has been associated with several autoimmune diseases, such as SLE. The aim of this study was to investigate how dysregulated miRNAs activate inflammatory pathways in SLE.

Methods: According to our keywords "Systemic Lupus Erythematosus, microRNAs, inflammation", we searched PubMed, Google scholar, and Scopus.

Results: MiR-146a, a negative modulator of the immune response, is downregulated in SLE patients. Proinflammatory cytokines like IL-21, which are involved in the pathogenesis of SLE, are expressed more frequently as a result of this downregulation. In addition, miR-148a and miR-126 are both shown to be overexpressed in T cells of SLE patients, resulting in T cell autoreactivity which triggers inflammatory pathways. Also, overexpression of miR-30a in SLE patients has been found to accelerate B cell proliferation and increase IgG production which is an important factor in inflammatory pathways. Moreover, it has recently been discovered that the miR-302d gene is downregulated in the monocytes of SLE patients. As a result, the type I IFN pathway is overactivated, which is a pathogenic factor for the uncontrollable production of inflammatory cytokines throughout the progression of SLE. It has also been demonstrated that other miRNAs, including miR-21, miR-10a, and miR-148a, are dysregulated in SLE and can induce inflammatory pathways.

Conclusion: Dysregulated miRNA expression play critical role in triggering inflammatory pathways in SLE. Targeting these dysregulated miRNAs could be a promising approach for developing novel treatments for SLE and other autoimmune disorders.

Keywords: Systemic Lupus Erythematosus, microRNAs, inflammation

E-cadherin (CDH1) gene single nucleotide polymorphisms and risk of endometriosis in Iranian women papulation

Amirhossein Khosrozadeh Ghomi[1], Mohammad Jafari[2], Ali-Akbar Delbandi[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Cellular and Molecular Research Center, Gerash University of Medical Science, Gerash, Iran

Backgrounds: Endometriosis is a chronic gynecological disease that is defined as a condition in which endometrial-like tissue can be observed outside of the uterus. Although the exact genes implicated in endometriosis remain unclear, Genetic research suggests that E-Cadherin regulates a wide range of adhesion in immunological reaction and plays an important anti-metastasis role. This study aimed to investigate any potential links between endometriosis and E-cadherin gene polymorphism.

Methods: Two important functional SNPs, the - 347G/GA (rs5030625) and - 160C/A (rs16260), exist upstream from the CDH1 transcriptional start site and would significantly influence transcriptional activity. This study involved one hundred and sixty-six Iranian women of reproductive age who underwent gynecological laparoscopy surgery. In 120 of these samples, endometriosis was identified however, the illness was ruled out histologically and laparoscopically in 46 of these individuals, who make up our control group. After extracting DNA from blood samples, we used the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method to check the mentioned polymorphisms.

Results: The genotypic frequency of the -160 and -347 polymorphisms did not significantly differ between the control and endometriosis groups. (Respectively p=0.48 and p=0.96; 95% CI)

Conclusion: This finding suggests that genetic polymorphism in the E-cadherin gene domain probably does not have significant effects on the development and progression of endometriosis, even though the functional link of E-cadherin polymorphisms is unclear.

Keywords: E-Cadherin, Endometriosis, Single nucleotide polymorphism, PCR-RFLP, Genotypic frequency

Effects of FcγRIIB and FcγRIIIA gene polymorphisms on systemic lupus erythematous disease activity index

Hossein Motedayyen[1], Farshid Fathi[2]

1. Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran
2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Systemic lupus erythematous (SLE) disease is a chronic autoimmune disease with unknown etiology that can involve different organs. Polymorphisms in Fcγ receptors have been identified as genetic factors in susceptibility to SLE. This study was aimed to investigate effects of two single nucleotide polymorphisms (SNPs) within FcγRIIB and FcγRIIIA genes on systemic lupus erythematous disease activity index (SLEDAI) in an Iranian population.

Methods: Blood samples were obtained from 80 SLE and 95 healthy individuals. Red blood cells were lysed and genomic DNAs were extracted using salting-out method. Genotype determinations of FcγRIIB and FcγRIIIA SNPs were performed by Real-time PCR-HRM.

Results: Our findings indicated TT and GG genotypes were the common genotypes of FcγRIIB and FcγRIIIA SNPs in SLE patients, respectively. There were no significant differences in genotype and allele frequencies of FcγRIIB and FcγRIIIA SNPs in SLE and healthy subjects. However, the frequencies of genotypes and alleles of FcγRIIB and FcγRIIIA SNPs were significantly associated with some clinical manifestations used to determine SLEDAI ($p< 0.001–0.5$).

Conclusion: Based on these findings, the frequencies of genotypes and alleles of FcγRIIB and FcγRIIIA SNPs may be useful in determining SLEDAI in the Iranian population.

Keywords: Systemic lupus erythematous, Fcγ receptor IIB, Fcγ receptor IIIA, SLE disease activity index, Polymorphism.

Evaluation of CCR5Δ32 Polymorphism in Patients with Systemic Lupus Erythematosus and Healthy Individuals

Seyedeh Reihaneh Hashemi[1], Zahra Heidaifard[2]

1. Student research committee, Iran.
2. Department of Virology, Iran.

Background: Chemokines play a role in the accumulation of inflammatory cells and have interaction with chemokine receptors on the surface of these cells. Increased levels of chemokines and chemokine receptors have been found in several autoimmune diseases. C-C chemokine receptor type 5 (CCR5) is a chemokine that expressed on the surface of T-cells. A 32-bp deletion in the coding region of the CCR5 (CCR5Δ32) leads to production of an incomplete protein which has been reported to have a protective effect on the development of several autoimmunity. Some studies indicated that mutation have protective effects on patients who deal with systemic lupus erythematosus, but has not been confirmed in other studies. As the importance of genetic predisposition in the prognosis of lupus, in this study we examined the frequency of CCR5Δ32 mutation in SLE patients and healthy individuals from the Golestan Province, Iran.

Methods: Whole blood samples were taken from 80 patients admitted to Shahid Sayyad Shirazi hospital and 80 healthy controls (from a blood bank) in the Golestan Province. Clinical and demographic data were collected through standard questionnaires and medical records. Serum C3 and C4 levels (mg/dl) were measured by nephelometry. Antibodies against double-stranded DNA (anti-dsDNA) were quantified using enzyme-linked immunoassay. Polymerase chain reaction (PCR) was used for amplification of CCR5 gene and CCR5Δ32 genotyping.

Results: Not only there was no statistically detectable difference in the genotype frequency between the SLE patients and healthy controls, but no significant association between the CCR5 status and clinical signs between these groups. Also, the homozygosity for CCR5Δ32 was not seen in both controls and patients.

Conclusion: Our data showed that the CCR5Δ32 polymorphism has no correlation with SLE in our study population. Besides, the frequency of the Δ32 in SLE patients and healthy ones does not follow the Hardy-Weinberg equilibrium.

Keywords: CCR5, Homozygote CCR5Δ32, Heterozygote CCR5Δ32, CCR5Δ32 allele

Evaluation of Epstein-Barr virus genes expression in peripheral blood mononuclear cells with Multiple Sclerosis patients under treatment

Faezeh Mehdizadeh[1], Behzad Baradaran[2], Mehrdad Pashazadeh[3]

1. Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Tabriz, Iran
2. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Immunology, Faculty of Medicine, Islamic Azad University, Tabriz, Iran

Background: Multiple sclerosis (MS) is one of the leading causes of severe physical disability in young people and the most common chronic autoimmune disease of the central nervous system. Various factors, such as genetics and environmental factors, can cause this disease. Epidemiological evidence has identified Epstein-Barr virus (EBV) infection as a prerequisite for the development of MS, but the mechanisms of this association are unknown. Therefore, the aim of this study is to analyze EBV genes in peripheral blood mononuclear cells (PBMC) of patients with MS, which can provide new information about this disease and help identify biomarkers.

Methods: Samples were selected from pathologically and clinically confirmed MS patients. Also, the control group was selected from healthy and asymptomatic people in terms of neurological symptoms. PBMC were collected from patient samples, and then the expression of several EBV genes associated with viral infection was determined by Real-Time PCR. Expression data were analyzed based on normal or non-normal data distribution using univariate and multivariate statistical methods.

Results: Several EBV-related genes were expressed in PBMC cells of MS patients. By statistical analysis, slight differences in the expression of related genes were observed. Also, by using Real-Time PCR, EBV transcript was detected in several patients.

Conclusion: The analysis of several EBV genes in PBMC cells of MS patient samples using Real-Time PCR can obtain new information about the complex interaction between the underlying biological processes of MS disease.

Keywords: Multiple Sclerosis, Epstein-Barr virus, Peripheral Blood Mononuclear Cells, Gene expression

Evaluation of Human herpes virus type 6 (HHV-6) genes expression in peripheral blood mononuclear cells with Multiple Sclerosis patients under treatment

Ghazal Pourbeiragh[1], Mehrdad Pashazadeh[2], Behzad Baradaran[3]

1. Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Tabriz, Iran
2. Department of Immunology, Faculty of Medicine, Islamic Azad University, Tabriz, Iran
3. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Multiple sclerosis is the most common chronic autoimmune disease of the central nervous system. Various factors such as genetics and environmental factors can play a role in causing this disease. Virus-related infections have been introduced as risk cofactors for MS, including human herpes virus type 6. This study aims to study HHV-6 genes in the peripheral blood mononuclear cells of patients with MS undergoing treatment by investigating the expression level of the genes of this disease in addition to active nerve plaques, at the level of blood cells.

Methods: The samples of the case group were selected from patients with MS who were confirmed pathologically and clinically. Also, the control group was selected from healthy and asymptomatic people with neurological symptoms. After isolating peripheral blood mononuclear cells from patient samples and extracting RNA from these cells, complementary single-stranded DNA is prepared from all samples using a cDNA synthesis kit to prepare for a Real-Time PCR test. Finally, by using the appropriate primer to examine the expression of HHV-6 genes, the relative expression level of this gene is obtained using the GAPDH internal control and compared between patient groups.

Results: In this study, several genes related to HHV-6 were expressed in PBMC cells of MS patients. By statistical analysis, slight differences in the expression of related genes were observed.

Conclusion: The results show that a subset of patients with RRMS experience HHV-6 active infection, and there likely is an association between the viral active replication and relapses; therefore, HHV-6 active infection may imply a greater risk of exacerbations in a subgroup of patients with RRMS.

Keywords: Multiple Sclerosis, Human herpes virus type 6 (HHV-6), peripheral blood mononuclear cells

Evaluation of miR-211-5p overexpression effect on PERK/ATF4/CHOP pathway and apoptosis related genes in FLS cells of rheumatoid arthritis

Maryam Farghadan[1], Ahmad Zavaran-Hosseini[2], Elham Farhadi[3], Arash Sharafat Vaziri[4], Mohammad Naghi Tahmasebi[4], Ahmadreza Jamshidi[5], Mahdi Mahmoudi[6]

1. Human and Animal Cell Bank, Iranian Biological Resource Center, ACECR, Tehran, Iran
2. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran, Inflammation Research Center, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Orthopedics, Division of Knee Surgery, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
5. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran
6. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran, Inflammation Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Fibroblast-like synoviocytes (FLSs) play a key role in the pathogenesis of rheumatoid arthritis (RA). Endoplasmic reticulum (ER) stress and dysregulation of unfolded protein response (UPR) is involved in resistance to apoptosis of RA-FLSs. Given the role of miR-211 in ER stress-induced apoptosis, we investigated the effect of miR-211-5p overexpression on PERK branch of ER stress and apoptosis related genes in RA-FLSs.

Methods: After FLSs isolation from synovial tissues of trauma and RA patients, phenotype characterization of the cells was accomplished by immunofluorescence staining and flow cytometry. miR-211-5p and mRNA expression of the selected genes involved in the PERK pathway and apoptosis regulation were measured in RA, trauma, and thapsigargin treated RA-FLSs. Afterward, miR-211-5p and mRNA levels of the studied genes were evaluated in thapsigargin treated RA-FLSs following miR-211-5p overexpression.

Results: The expression of miR-211-5p, PERK, BAX, and BCL2 showed no differences between RA and trauma FLSs. However, the expression of ATF4 and BCL-XL showed a significant increase in trauma compared to RA-FLSs. In addition, the levels of CHOP and MCL1 indicated a significant increase in RA-FLSs compared to trauma. Thapsigargin treatment significantly increased the expression of PERK, ATF4, and CHOP in RA-FLSs with no effect on miR-211-5p, BAX, BCL2, BCL-XL, and MCL1. Furthermore, thapsigargin treatment following miR-211-5p overexpression in RA-FLSs showed a significant increase in levels of miR-211-5p with no change on the expression of CHOP and apoptotic genes.

Conclusion: Based on the results, it seems that there is ER stress in RA and trauma FLSs. In addition, stimulation of ER stress in RA-FLSs and overexpression of miR-211-5p in stimulated RA-FLSs did not alter the expression of selected genes in apoptosis regulation. However, more investigations are necessary to determine the ER stress role in apoptosis regulation in RA-FLSs.

Keywords: ER stress, Fibroblast-like synoviocytes (FLSs), miR-211-5p, Rheumatoid arthritis (RA)

Evaluation of NF-κBIαgene expression in PBMCs of patients with coronary artery disease

Shakiba Kazemian[1], Nader Bagheri[1], Ali Rafiei[1], Fatemeh Azadegan-Dehkordi[1], Arsalan Khaledifar[1]

1. Shahrekord University of Medical Sciences, Shahrekord, Iran.

Background: Atherosclerosis is a chronic inflammatory disease of the arterial intima and is the primary cause of coronary artery disease (CAD) which is the leading cause of death worldwide. Inflammation has long been recognized as a key factor in the progression of CAD. Some crucial inflammatory genes are regulated post-transcriptionally in this pathway, which may aid in the formation of atherosclerotic plaques. Therefore, the aim of this study was to evaluate the expression of NF-κBIα in the peripheral blood mononuclear cells (PBMCs) of the CAD group compared with the control group.

Methods: 168 subjects (84 CAD subjects and 84 control subjects) were included in this study and the expression level of NF-κBIαin PBMCs was measured using the real-time PCR technique.

Results: Comparison of the CAD group with the control group indicated a significantly reduced expression level of NF-κBIα Also, there was a statistical correlation between the numbers of clogged arteries with the expression level of NF-κBIαin the CAD group.

Conclusion: In patients with CAD, decreased expression of the NF-κBIαgene may have a role in the progression of atherosclerotic plaques.

Keywords: Coronary Artery Disease, Atherosclerosis, Inflammation, NF-κBIα

Evaluation of the genetic diversity of KIR genes in patients with endometrial cancer

Kimia Mirshekari[1], Mohammad Javad Fattahi[1], Zahra Shiravani[2], Narges Shafiey[2], Abbas Ghaderi[3]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Gynecology Oncology division, Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Endometrial cancer (EC) is the cause of more than half of all genital cancers in women with an increasing incidence in different countries. Natural killer cells (NK cells) are kinds of innate immune cells that are controlled by sets of receptors such as killer cell Ig-like receptors (KIRs) which can inhibit or activate NK cells. In this study we evaluated the diversity and genetic association of KIRs in confirmed cases of endometrial cancer compared to healthy women.

Methods: Demographic and histopathologic data gathered in a questionnaire for 151 women with EC and 167 age/race matched healthy women. DNA was extracted from blood samples and 16 KIR genes along with two variants of KIR2DS4 (KIR2DS4fl and KIR2DS4del) were genotyped by using sequence specific primers-polymerase chain reaction (SSP-PCR) method.

Results: A comparison between cases and controls revealed that although there were not any significant differences in haplotype A associated genes and also the variants of KIR2DS4 (p>0.05), haplotype B associated genes such as KIR2DS2 and KIR2DL2 decreased significantly in EC patients in comparison with healthy controls (p=0.03 and p=0.01, respectively). Furthermore, we found that EC mostly developed in cases with AA genotype, however, the carriers of Bx and C4T4 genotypes were resistance to EC.

Conclusion: Our results revealed that KIR2DS2 and KIR2DL2 along with Bx and C4T4 genotypes have a protective impact on developing endometrial cancer in Iranians.

Keywords: endometrial cancer (EC), genetic, killer cell Ig-like receptors (KIRs), genetic variation.

Frequency of HLA-B allelic groups in Arab ethnic group of Iran

Fatemeh Sabaghi[1]

1. Department of Hematology and Blood Banking, Faculty of Allied Medicine, Iran University of Medical Science, Tehran, Iran

Background: Human leukocyte antigens display the highest degree of polymorphism in the genome of different species. The distribution of HLA alleles varies among different ethnicities. Determination of frequency of HLA alleles in different ethnic groups will be useful in the expansion of centers for the registration of stem cell donors and stem cell transplantation studies.

Methods: In this study, DNA was extracted from the whole blood samples of unrelated stem cell donors. Ethnic and HLA information of Arab (n = 370) ethnic population was analyzed and the relationship between the allele and ethnicity was examined and a significant relationship between the frequencies of alleles in the studied ethnicity were evaluated by chi-square statistical method. The allele groups of HLA-B were determined at low resolution level (2-digit) by polymerase chain reaction-sequence-specific primer (PCR-SSP) method.

Results: Allelic frequencies were obtained for 370 Arab stem cell donor candidates were obtained as below HLA- B*37, HLA- B*39, HLA- B*42 less than 1%. In allelic groups of HLA- B*40, HLA- B*49, HLA- B*53, the allele frequency displays <3%. The most common alleles identified for HLA-B were HLA-B*35 HLA-B*51, and HLA-B*50, with the highest allelic frequencies 11.4%, 9.3% and 7.7%, respectively in the studied Arab population.

Conclusion: The frequency of alleles in the Arab group, one of the Iranian races, can help in obtaining the information required in the HLA registry. The chance of finding an unrelated donor with similar HLA is higher in people of the same ethnicity, which is effective in the success of the hematopoietic stem cell transplantation.

Keywords: HLA, Iran, Arab ethnic group

Gene therapy in autoimmune diseases

HLA-DRB1 and –DQB1 alleles and haplotypes frequencies among children with T1D and their siblings in Hamadan province, West-Iran.

Ata Shirizadeh[1], Zahra Razavi[1], Mahdi Behzad[1], Ghasem Solgi[1]

1. Hamadan University of Medical Sciences, Hamadan, Iran.

Background: This family study aimed to determine the HLA-DRB1 and DQB1 genotypes and haplotypes among children with type 1 diabetes (T1D) and their siblings and to assess the genetic risk for disease among healthy siblings.

Methods: A total of 70 T1D patients were recruited to this family and case-control study. Genotypes of HLA-DRB1 and -DQB1 allele's families for all participants were determined by using low- and high-resolution PCR-SSP method. HLA class II genotypes and haplotypes for all patients and 125 siblings as well as 150 healthy ethnically matched controls were analyzed.

Results: The most susceptible alleles for disease were HLA-DRB1*03:01, DRB1*04:02, DQB1*02:01 and DQB1*03:02 and protective alleles were HLA-DRB1*11:01, *13:01, *14:01 and DRB1*15 as well as HLA-DQB1**06:02 and *06:03. Haplotype analysis revealed that T1D patients had higher frequencies of DRB1*03:01–DQB1*02:01 (p<0.0001) and DRB1*04:02–DQB1*03:02 (p<0.001) and lower frequencies of DRB1*11:01–DQB1*03:01 (p=0.006), DRB1*13:01–DQB1*06:03 (p=0.005) and DRB1*15–DQB1*06:01 (p=0.01) haplotypes compared to healthy controls. Heterozygote combination of both susceptible haplotypes (DR3/DR4) confers the highest risk for T1D (RR=16.5 p=0.003). A comparison of patients with T1D and their siblings proposed a dose effect of susceptible and protective DRB1~DQB1 haplotypes on disease onset.

Conclusion: Our findings not only confirm earlier reports from Iranians but also, are in line with Caucasians and partly with Asians and some African T1D patients. Siblings' analysis revealed a possible risk prediction method for T1D based on HLA-DRB1 and DQB1 alleles and haplotypes determination.

Keywords: Type 1 Diabetes, HLA-DRB1 and DQB1, allleles.

HLA-DRB1 and –DQB1 alleles and haplotypes frequencies among children with T1D and their siblings in Hamadan province, West-Iran.

Ata Shirizadeh[1]

1. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

Background: This family study aimed to determine the HLA-DRB1 and DQB1 genotypes and haplotypes among children with type 1 diabetes (T1D) and their siblings and to assess the genetic risk for disease among healthy siblings.

Methods: A total of 70 T1D patients were recruited to this family and case-control study. Genotypes of HLA-DRB1 and -DQB1 allele's families for all participants were determined by using low- and high-resolution PCR-SSP method. HLA class II genotypes and haplotypes for all patients and 125 siblings as well as 150 healthy ethnically matched controls were analyzed.

Results: The most susceptible alleles for disease were HLA-DRB1*03:01, DRB1*04:02, DQB1*02:01 and DQB1*03:02 and protective alleles were HLA-DRB1*11:01, *13:01, *14:01 and DRB1*15 as well as HLA-DQB1**06:02 and *06:03. Haplotype analysis revealed that T1D patients had higher frequencies of DRB1*03:01–DQB1*02:01 (p<0.0001) and DRB1*04:02–DQB1*03:02 (p<0.001) and lower frequencies of DRB1*11:01–DQB1*03:01 (p=0.006), DRB1*13:01–DQB1*06:03 (p=0.005) and DRB1*15–DQB1*06:01 (p=0.01) haplotypes compared to healthy controls. Heterozygote combination of both susceptible haplotypes (DR3/DR4) confers the highest risk for T1D (RR=16.5 p=0.003). A comparison of patients with T1D and their siblings proposed a dose effect of susceptible and protective DRB1~DQB1 haplotypes on disease onset.

Conclusion: Our findings not only confirm earlier reports from Iranians but also, are in line with Caucasians and partly with Asians and some African T1D patients. Siblings' analysis revealed a possible risk prediction method for T1D based on HLA-DRB1 and DQB1 alleles and haplotypes determination.

Keywords: Type 1 Diabetes, HLA-DRB1 and DQB1, allleles.

HLA-DRB1*04:05 and -DQB1*06:02 may predict poor response to treatment in rheumatoid arthritis patients

Ghasem Solgi[1], Ahmad Tahamoli-Roudsari[2], Ashkan Rasuli-Saravani[3], Zahra Basiri[4], Mehrdad Hajilooi[5]

1. Hamadan University of Medical Sciences, Hamadan, Iran.

Background: The present study was conducted to explore the distributions of susceptibility and protective HLA-DRB1 alleles for rheumatoid arthritis (RA) and to ascertain the association of HLA-DRB1 alleles with the pattern of response to treatment; poor response (PR) and good response (GR) to therapy.

Methods: A total of 167 RA patients were recruited to this prospective cohort and case-control study between September 2016 and December 2018. Disease activity and clinical response to therapy were evaluated based on DAS28 score at months 3, 6 and 9 post treatment. Also, 150 ethnic-matched healthy subjects were included as controls. Genotypes of HLA-DRB1 and -DQB1 alleles families for all participants were determined by using low- and high-resolution PCR-SSP method.

Results: 109 out of 167 patients (65.2%) carried at least one HLA-Shared Epitope (SE) alleles (DRB1*01, *04, *10 and *14) and 106 patients (63.5%) were positive for ACPAs. 73 cases (43.7%) were positive for both HLA-SE alleles and ACPAs. 114 patients were determined with good response (GR) and 53 patients with poor response (PR) to treatment. Higher significantly frequencies of DRB1*04 and DRB1*10 alleles families and lower frequencies of DRB1*13 allele group were observed in the RA patients versus healthy controls. Also, higher frequencies of DRB1*04:05 (p=0.03, OR=0.354) and DQB1*06:02 (p=0.01, OR=0.218) alleles were found in the PR patients compared to GR patients. Whereas, DRB1*11 allele family was more frequent in the GR than PR patients (p=0.07 and p=0.04 respectively). ACPAs-positivity was more frequent in the SE-positive compared to SE-negative RA patients (70.7% vs. 56.4%, p=0.07).

Conclusion: Our findings further indicate the relationship between HLA-SE alleles and ACPAs development and the potential link between HLA SE/non-SE alleles and therapeutic responses in RA patients.

Keywords: Rheumatoid Arthritis, HLA-DRB1, Shared Epitope.

IL-10 Promoter gene polymorphism (rs1800896) patients with colorectal cancer

Seyed Sajad Alavimanesh[1], MohammadRasul ZareiNejad[2], Zahra Mansourabadi[1], Zahra Mojtahedi[2], Zahra Faghih[2]

1. Shiraz Institute for Cancer Research, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Medical Genetics, School of Medicine, Shahrekord University of Medical Science, Shahrekord, Iran

Background: Chronic inflammation as one hallmark of cancer is mediated by different inflammatory factors and cytokines which play a crucial role in the development and multiplication of cancerous cells. Interleukin (IL)-10, produced by a wide range of cells, is a highly pleiotropic cytokine which plays contradictory roles in cancer mainly as tumor-inhibiting mediator. It seems to facilitate tumor immune escape and elevated level of this cytokine has been shown to be associated with poor prognosis, the tumor growth and drug resistance. In the present study, we aimed to investigate a single nucleotide polymorphism (SNP) in the promoter of the IL-10 gene, rs1800896, with a possible effect on the expression level in patients with colorectal compared to healthy donors.

Methods: Blood samples were obtained from 121 patients newly diagnosed with colorectal cancer (mean age=55.74±14.069 years) and 119 age-sex matched healthy individuals with no history of cancer and autoimmune diseases. DNA was extracted and genotyped by ARMS-PCR method using specific primers for rs1800896. SPSS software version 23 was applied for data analysis and P-value less than 0.05 was considered significant.

Results: Our analysis indicated that the frequency of AA genotypes of rs1800896 was significantly higher in the controls compared to patients (86.1% vs. 50.9%), while AG genotypes were more frequent in the patients (45.6% vs. 12.2%) (p<0.0001).

Conclusions: Since the A allele has been proposed to be associated with lower expression of IL-10, our data collectively suggest a protective role for this allele in colorectal cancer.

Keywords: Colorectal cancer, IL-10, Gene polymorphism, rs1800896

Immunogenic epitope analysis of Iranian cobra (Naja oxiana) snake venom using peptide displayed phage library

Nastaran Mohseni[1], Fatemeh Kazemi-Lomedasht[1]

1. Biotechnology Research Center, Venom and Biotherapeutics Molecules Laboratory, Pasteur Institute of Iran, Tehran, Iran

Background: Snake venom pharmacologically comprises active components. This study aimed to identify mimotopes of Naja oxiana. Phage display peptide library, Ph.D.C7C, was utilized and biopanning was carried out on immobilized horse antibodies.

Methods: Three rounds of selection were performed on purified antibodies. Single phages randomly selected from the third round of biopanning were amplified and subjected to DNA sequencing.

Results: DNA sequencing results revealed 35 peptides with unique sequences.

Conclusion: Obtained peptides represent some linear mimicking epitopes of venom antigens that involve in interaction with antibodies and could be used for further immunological and venomics study.

Keywords: Epitope mapping, mimotope, phage display

Investigation of Correlation Pro-inflammatory Cytokines (IL-6, IL-8, TNF-a) Levels with rs 1049174 G>C Gene Polymorphism in Ductal Carcinoma Breast Cancer Women

Mahnaz Sadat Zahraei[1]

1. Shahid Sadoughi University of Medical Sciences-Yazd, Iran

Background: Breast cancer is the second leading cause of cancer death in women worldwide. Although the death rate from breast cancer has been steadily decreasing since 1989, the age at which this malignancy occurs has decreased. In Iran, more than 6 thousand new cases of breast cancer are diagnosed yearly. NKG2Dreceptor on NK cells is one of the main activator immune receptors. In human NKG2D gene has three single nucleotide polymorphisms (SNP) (rs1049174 G>C). New research showed that CC genotype reduces NKG2D affinity and is caused by dysfunction in NK cells. Aim of this study, survey of correlation NKG2D SNPs with the severity of malignancy and the pro-inflammatory Cytokines (IL-6, IL-8, TNF-a) and in breast cancer patients.

Methods: In this Case- Control research, 100 breast cancer patient (Ductal Carcinoma) and 100 healthy women were selected from breast cancer care center (Iran, Yazd). RFLP-PCR assay using with specific primers forNKG2D gene polymorphism (SNP) (rs 1049174 G>C) was performed, also serumic pro-inflammatory cytokines (IL-6, IL-8, TNF-a) measurement by ELISA kit. Statistical analysis was done using SPSS version 22, GraphPad prism version 8 software.

Results: According to our analysis, frequency of CC and GG, GC, CC genotypes in the patients were 68%,18%,4% in comparison to 40%,43%,17% respectively in healthy women (p<0.05). CC genotype has more susceptibility to breast cancer versus control group (CI=95%, 1.31-2.24, OD=1.7). Also, the amounts of pro-inflammatory Cytokines IL-6 (28.84±15.12 pg/ml), IL-8 (113.05±19.02 pg/ml), TNF-a(49.68±29.93 pg/ml) in the ductal carcinoma breast cancer CC genotype have increased significantly statically compared to the other genotype (GG, GC) and the control groups (p<0.05).

Conclusion: Results of this research demonstrated that homozygote CC genotype SNP (rs1049174 G>C) can be increased the risk of ductal carcinoma breast cancer. Since SNP (rs1049174 G>C) genotype is placed on the theNKG2D receptor of the NK cells, which is the first line of immune system defense against the cancer cells, women with the CC genotype have a low affinity for binding to the ligand (MICA/MICB), resulting in increased inflammation through the production of pro-inflammatory cytokines (IL-6, IL-8, TNF-a) cytokines. However, more extensive studies are necessary to find the exact mechanisms of increased inflammation in this pathway.

Keywords: breast cancer; ductal carcinoma; nkg2d receptor; polymorphism; Snp; proinflammatory cytokines

NOD2 Gene Polymorphism (rs2066844) and Susceptibility to Pulmonary Tuberculosis in Zahedan, Southeast Iran

Mahdi Atabaki[1], Ebrahim Alijani[1]

1. Clinical Immunology Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

Background: Pulmonary tuberculosis (PTB) in human societies is believed to be a major health threat and is considered as an important cause of morbidity and mortality worldwide. Nucleotide-binding oligomerization domain 2 (NOD2) is one of the pathogen recognition receptors (PRRs) that play its function in account of the recognition of peptidoglycans in bacterial cell wall. NOD2 gene polymorphisms could influence the levels of gene expression and may attribute to infection.

Methods: The present case-control study was conducted on 152 patients with PTB and 162 healthy subjects to determine whether the NOD2 rs2066844 polymorphisms is associated with PTB. This polymorphism was determined utilizing PCR-restriction fragment length polymorphism (PCR-RFLP) after designing specific primers.

Results: The results of our study showed that the frequency of CC genotype in the case and control groups was 152 (100%) and 162 (100%), respectively, and the other two genotypes (TT and CT) were not observed in our population.

Conclusion: we noticed that the NOD2 rs2066844 polymorphism is not a susceptibility factor for PTB in an Iranian population sample. It is suggested that this polymorphism be evaluated in a larger population to obtain more reliable results.

Keywords: Pulmonary tuberculosis, NOD2, Gene polymorphism

Peripheral distributions of IL-4-producing CD4 + T cells and CD4 + CD25 + FoxP3 + T cells (Tregs) in rheumatoid arthritis patients with poor response to therapy are associated with HLA shared epitope alleles

Ashkan Rasouli-Saravani[1], Ghasem Solgi[2]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

Background: Specific profiling of CD4 + T cell subsets in the circulation and inflamed joints of rheumatoid arthritis (RA) patients may have therapeutic implications.

Methods: This study aimed to evaluate the peripheral distributions of Th2 and Treg cells in relation to HLA-shared epitope (SE) alleles in patients with good response (GR) and poor response (PR) to treatment. The frequencies of IL-4-producing CD4 + T cells (Th2) and CD4 + CD25 + Foxp3 + T cells (Tregs) were determined by flow cytometry in 167 RA patients including 114 GR and 53 PR cases. CD4 + T cell subsets were also analyzed based on HLA-SE.

Results: One hundred nine of 167 patients were positive for HLA-SE. Higher frequencies of Th2 (p= 0.001) and Treg cells (p= 0.03) were found in the patients versus controls. Increased and decreased frequencies of Th2 and Treg cells were observed in the PR versus GR patients, respectively (p= 0.003 and p= 0.004). Higher proportions of Th2 cells were observed in the SE+RA versus SE−RA (p= 0.001). Treg cells frequencies decreased in the SE+RA versus SE−RA (p = 0.03) and in SE+ACPA+RA versus SE−ACPA−RA (p = 0.02). SE+PR patients showed higher proportions of Th2 cells than SE−PR patients (p= 0.02 and p= 0.01).

Conclusion: Analysis of the CD4 + T cell subsets profiles in conjunction with the genetic background can be useful for precise therapeutic response monitoring in RA patients.

Keywords: Rheumatoid arthritis, HLA-shared epitope, Th2, Treg

Relationship analysis of the miR-196a2 polymorphism (rs11614913) with colorectal cancer risk in southern Khorasan, eastern Iran

Malaksima Ayadilord[1], Mohsen Naseri[2]

1. Department of Medical Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.

Background: It has been considered that colorectal cancer (CRC) is the fourth most common cause of cancer death with multifactorial characteristics and genetic precedent can provide a comprehensive sight of its etiology. Single nucleotide polymorphisms (SNPs) in microRNAs (miRNAs /miRs) sequences are considered as prognostic markers with high specificity for CRC detection and prevention. Recent researches have indicated that rs11614913 is the most common SNP related to the vulnerability to many of the cancers. This case-control study aims to quantify the correlation between rs11614913 in miR-196a2 and CRC in the southeast Iranian population.

Methods: To identify the role of rs11614913 SNP in susceptibility to CRC, this case-control study is done on CRC patients (n=52) and healthy persons (n=120) of the same organization with a uniform gender and age dispensation compared to CRC patients and no prior background of any kind of cancer by PCR-RFLP technique. Statistical analysis is carried out utilizing SPSS (version 16).

Results: After the comparison of genotype and allele distribution of this SNP among case and control groups, it has not shown any statistically significant findings (p>0.05).

Conclusion: We have not discovered a statistically significant association between rs11614913 polymorphism in miR-196a2 and the risk of CRC in the southeast Iranian society.

Keywords: RFLP, miR-196a2, Polymorphism, Colorectal cancer

Review on the gene expression profile of pemphigus vulgaris: Emphasis on the most differentially expressed genes

Shamim Shahmohammadi[1], Fatemeh Karami[2]

1. Department of Biology, School of Converting Sciences and Technologies, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Medical Genetics, Applied Biophotonics Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran

Background: Pemphigus vulgaris (PV) is an autoimmune disease which is characterized by autoantibodies against desmosomes of epithelium adhesion compartment leading to skin blistering and consequent fatal complications. Identification of the main gene expression pattern can open the window toward targeted and more efficient therapy for PV patients.

Methods: We explored the internet in Google Scholar, PubMed, PubMed Central, MEDLINE, EMBASE, and Web of Science using main keywords including Pemphigus vulgaris, gene expression, miRNA and disease severity. All the manuscripts from 2010 till the end of 2023-04-01 were included in the present study.

Results: The role of Th17 expression and subsequent IL-17 production was found to be associated with desmoglein1/3-specific autoantibodies in either case control or functional studies. Among miRNA, overexpression of miR-338-3p was demonstrated to be associated with PV severity with a higher coefficient than desmoglein1/3 antibodies.

Conclusion: Owing to the immune-modulatory roles of miR-338-3p and Th17, further functional studies are warranted to determine their exact contribution in PV pathogenesis. Shedding light on their roles not only make them as powerful diagnostic markers of PV, but also will afford novel personalized therapy for the patients.

Keywords: Pemphigus vulgaris, gene expression, miR-338-3p, Th17

The effect of APR246 small molecule on the expression of ER stress genes related to apoptosis in rheumatoid arthritis fibroblast-like synoviocytes

Maryam Farghadan[1], Elham Madreseh[2], Maryam Akhtari[3], Mohsen Rokni[4], Mina Sadeghi Shaker[4], Nooshin Ahmadzadeh[3], Maassoumeh Akhlaghi[3], Mohammad Naghi Tahmasebi[5], Arash Sharafat Vaziri[5], Ahmadreza Jamshidi[6], Elham Farhadi[7], Mahdi Mahmoudi[4]

1. Human and Animal Cell Bank, Iranian Biological Resource Center, ACECR, Tehran, Iran
2. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran
3. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran, Inflammation Research Center, Tehran University of Medical Sciences, Tehran, Iran
4. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran, Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Orthopedics, Division of Knee Surgery, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
6. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran,
7. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran, Inflammation Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: In rheumatoid arthritis (RA), fibroblast-like synoviocytes (FLSs) are the major pathological cells that display tumor-like behavior including aggressive phenotype, hyper-proliferation, and apoptosis resistance. The FLSs in RA patients are resistant to the endoplasmic reticulum (ER) stress-induced apoptosis. In this study, we evaluated the effect of APR246 (also named PRIMA-1MET) as a mutant p53 reactivator on ER stress genes related to apoptosis in RA-FLSs.

Methods: The FLSs samples were obtained from synovial tissues of RA patients (n=10). After characterization, the cells were treated with different doses of APR246. The rate of apoptosis and cell survival was measured by flow cytometry and 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. Furthermore, the mRNA levels of the selected ER stress genes involved in apoptosis including BIP, CHOP, GADD34, NOXA, HSPA1B, BCL2, and MCL1 were analyzed by Real-time PCR method in RA-FLSs.

Results: The results demonstrated that APR246 treatment in RA-FLSs leads to decreased cell survival and induction of apoptosis in a dose-dependent manner. In addition, APR246 treatment significantly increased the mRNA expression of GADD34, CHOP, NOXA, and HSPA1B genes in RA-FLSs. Although APR246 treatment did not show any change in the mRNA expression levels of BIP, BCL2, and MCL1 genes in RA-FLSs.

Conclusion: Given the role of ER stress genes in RA-FLSs apoptosis, it seems that APR246 small molecule can be considered as a therapeutic approach in RA patients. However, further studies are needed to confirm these results.

Keywords: APR246, ER stress, Fibroblast-like synoviocytes (FLSs), Rheumatoid arthritis

Immunoinformatics & Insilico Immunology

Hardware Design to Accelerate Molecular Dynamics Simulation of mRNA Vaccines

Faezeh Sadat Mozneb[1], Parvaneh Asghari[2]

1. Islamic Azad University South Tehran Branch, Tehran, Iran.
2. Islamic Azad University Central Tehran Branch, Tehran, Iran.

Background: A newly designed mRNA vaccine requires precise molecular dynamics simulation to discover its behavior in human body prior to performing risky in vivo experiments. Such a precise discovery requires simulating millions of atoms for a simulation interval in the range of days. However, simulating millions of atoms for a simulation interval equal to 1 minute takes an execution time in the range of years on an ordinary computer.

Methods: In this research, we intend to design computer hardware to accelerate molecular dynamics simulation so that the execution time is reduced from years to a few days. The computer hardware consists of multiple FPGA boards that run in parallel and are connected to a desktop computer.

Results: We design and program the FPGA boards to achieve a high speed gain compared to GPU acceleration. The number of FPGA boards connected to a computer is not restricted so that we can increase the execution speed as much as we want. In this way, we do not need expensive supercomputers for large scale simulations.

Keywords: Molecular Simulation, mRNA, FPGA, Acceleration

Identification of an immune-related genes signature in lung adenocarcinoma to predict survival and response to immune checkpoint inhibitors

Zeinab Davoodi-Moghaddam[1], Farideh Jafari-Raddani[1], Shahram Kordasti[2], Davood Bashash[1]

1. Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Comprehensive Cancer Centre, School of Cancer and Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom

Background: Although advances in immune checkpoint inhibitor (ICI) research have provided a new treatment approach for lung adenocarcinoma (LUAD) patients, their survival is still unsatisfactory, and there are issues in the era of response prediction to immunotherapy.

Methods: Using bioinformatics methods, a prognostic signature was constructed and its predictive ability was validated both in the internal and external datasets (GSE68465). We also explored the tumor-infiltrating immune cells, mutation profiles, and immunophenoscore (IPS) in the low-and high-risk groups.

Results: As far as we are aware, this is the first study which introduces a novel prognostic signature model using BIRC5, CBLC, S100P, SHC3, ANOS1, VIPR1, LGR4, PGC, and IGKV4.1. According to multivariate analysis, the 9-immune-related genes (IRGs) signature provided an independent prognostic factor for overall survival (OS). The low-risk group had better OS, and the tumor mutation burden (TMB) was significantly lower in this group. Moreover, the risk scores were negatively associated with the tumor-infiltrating immune cells, like CD8+ T cells, macrophages, dendritic cells, and NK cells. In addition, the IPS were significantly higher in the low-risk group as they had higher gene expression of immune checkpoints, suggesting that ICIs could be a promising treatment option for low-risk LUAD patients.

Conclusion: The combination of these 9-IRGs not only could efficiently predict the overall survival of LUAD patients but also show a powerful association with the expression of immune checkpoints and response to ICIs based on IPS; hoping this model paves the way for better stratification and management of patients in clinical practice.

Keywords: lung adenocarcinoma, immune-related signature, immunotherapy, immune checkpoint inhibitor, tumor immune microenvironment

Mazdak Ganjalikhani Hakemi

Nooshin Ghadiri[1]

1. *Immunology of Ajums*

Background: miRNAs, non-coding RNAs, take part in different cellular proceedings. Dysregulation of different miRNAs has been reported in numerous disorders to date. Multiple sclerosis (MS) is one autoimmune disease with high prevalence in Iran and Th17 cells show that have an important role in its pathogenesis. In the current study, we aimed to predict the possible role of miR-34a and miR- 215in the process of controlling Th17 differentiation, and hence, their possible impact on the onset and progression of MS.

Methods: We pursued probable interactions of miRNAs and genes that participate in Th17 cells differentiation using the miRwalk database to predict miRNA-mRNA interaction.

Results: According to our findings, miR-134a and miR-215 were predicted that have a potential role in Th17 cells differentiation.

Conclusion: Conclusively, miR-34a and miR-215 may up-regulate Th17 cells of MS patients. Because bioinformatics data have shown that these miRNAs suppress negative regulatory genes in Th17 cells differentiation, so we suppose that down-regulation of these miRNAs could ameliorate MS symptoms. Thus, several therapeutic approaches may be considered for these miRNAs besides their application as valuable prognostic/diagnostic biomarkers in the detection of various stages of MS.

Keywords: Multiple sclerosis, miRNA, Th17 cells

A bioinformatics analysis for determine the role of miR-451a in immunogenic cell death amplification

Mahdieh Azizi[1], Sadra Salehi-Mazandarani[2], Marzieh Rezaei[1]

1. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
2. Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Immunogenic cell death (ICD) is a type of cellular death that is elicited in response to the specific types of anti-cancer therapies and enhanced the anti-tumor immune responses by the combination of antigenicity and adjuvanticity of dying tumor cells. There is a well-established interlink between endoplasmic reticulum stress (ERS) and ICD elicited by anti-cancer therapies. Most recent evidence supports that unfolded protein response (UPR)-associated miRNAs can be key players in the ERS-induced ICD. MiR-451a has been introduced as a tumor suppressor microRNA that can cause apoptosis of tumor cells by inducing endoplasmic reticulum stress; therefore, it seems that it can be considered a suitable target for ICD induction.

Methods: For investigating the possibility of occurrence and exacerbation of immunogenic death in cancer cell lines by miR-451a, were done bioinformatics analysis and the identification of target mRNAs interacting with miR-451a, via using multiMiR R package in the R Studio software. Enrichment analysis, consisting of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms were performed based on the mRNAs from the miRNA-mRNA network utilizing the Enrichr online database. A Protein-Protein Interaction (PPI) network was then constructed by STRING online database. Finally, hub genes of the PPI network (based on degree centrality) were identified by CytoHubba plugin in the Cytoscape software.

Results: Our results showed that “PI3K-Akt signaling pathway”, “pathways in cancer”, “TNF signaling pathway”, and “MAPK signaling pathway”, which correlate with UPR components and ERS induction, were among the significant signaling pathways related to the target genes of this miRNA. Furthermore, a protein-protein interaction (PPI) network was constructed, which revealed the involvement of the PPI-extracted hub genes in the regulation of proliferation and apoptosis.

Conclusion: We propose that miR-451a can be considered as a potential cancer therapy option for better induction of ICD in combination with other ICD inducers.

Keywords: Immunogenic cell death, Endoplasmic reticulum stress, Unfolded protein response, miR-451a, Cancer, Bioinformatics

A Comprehensive Systems Biology Approach to Understanding Immune System Function and Dysfunction: Advancing our Understanding of the Immune System through Data Integration

Seyyed Mohammad Yaghoubi[1], Behzad Baradaran[2]

1. Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: The complex immune system serves as a vital defence apparatus for the human body, offering formidable protection against the deleterious impacts posed by harmful microorganisms while ensuring proper internal equilibrium is maintained. It is of utmost importance to acknowledge the profound impact that this entity possesses and abstain from underestimating its intrinsic contribution to the contemporary situation. Instances of disturbances in immune system activity can result in diverse afflictions, including but not restricted to autoimmune complications, contagions, sensitivities, and oncogenic formations. To truly comprehend the molecular intricacies of both a functioning and dysfunctional immune system, we must adopt a systems biology approach that deftly integrates information from myriad sources spanning an assortment of organizational levels.

Methods: Data integration and system biology approaches were utilized to study the complex interactions between immune cells and molecules. The approaches endeavor to merge information from various origins and magnitudes to create an all-inclusive understanding of the immune system. An assortment of methodologies is employed to ascertain cellular biomarkers, pathways, and mechanisms linked with immune responses or afflictions.

Results: Data integration and systems biology approaches have provided novel insights into the immune system by identifying key players, interactions, pathways, and networks that are involved in health and disease. Numerous methods have been employed in scrutinizing various facets of the immune system inclusive of but not limited to innate immunity, adaptive immunity, host-pathogen interactions, vaccine responses as well as immunotherapy.

Conclusion: Ultimately, to comprehend the workings of the immune system and its irregularities, data integration, and systems biology techniques are imperative. These methodologies bear great promise in terms of discovering biomarkers, producing medications, and executing customized medical treatments within immunology. Nevertheless, certain hindrances exist at present that must be resolved along with future directions that require attention if we aspire to unlock their complete potential.

Keywords: Data integration, Systems biology, Immune system, Biomarker Discovery, Drug development, personalized medicine.

A novel allergy vaccine against the most allergenic grass pollen (Cynodon dactylon, Poa pratensis, Phleum pratense): an in-silico based study

Mina Noroozbeygi[1], Mina Mohammad-rezaei[1], Mohammad-Ali Assarehzadegan[1]

1. Immunology Research Center, Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Respiratory allergic reactions are IgE-mediated hypersensitivity. Pollen allergy is one of the main causes such reactions. Cynodon dactylon, Poa pratensis and Phleum pratense are three types of most allergenic grass.

Methods: Cyn d 1, Poa p 1 and Phl p 1 are known allergens of Cynodon dactylon, Poa pratensis and Phleum pratense respectively, which are used in this study using immunoinformatics methods to design a peptide allergy vaccine. The steps after finding the main allergens include sequences retrieval, T cell epitopes selection by IEDB and NetMHC, B cell epitopes selection by IEDB and BCpred, evaluation of antigenicity, allergenicity, toxicity, addition of linkers and adjuvant, secondary structure prediction, 3D structure modelling by GalaxyTBM, validation the model by ProSA-web, ERRAT, RAMPAGE, and exploring interaction of designed molecule with receptor by SwarmDock.

Results: The results of our study showed that 5KPGPNITATYGSKWL19 (for Cyn d 1), 27VPPGPNITATYGDKWL42 (for Poa p 1) and 50GKPTAAGPKDNGGA63 (for Phl p 1) are the most potential predicted B cell epitopes, while 193NDHYLALLVKYAAGD207 (for Cyn d 1), 214ESWGSIWRVDTPDKL228 (for Poa p 1) and 218AIWRIDTPEVLKGPF232 (for Phl p 1) are the most potential predicted T cell epitopes interacted with MHC allelic protein HLA-DRB1*11:01, HLA-DRB3*01:01 and HLA-DRB3*01:01 respectively, with the lowest IC50 value.

Conclusion: The designed multi-epitope vaccine can be used as a therapeutic or preventive vaccine for related grass allergens. But it is necessary to confirm the results of this study with immunological assays.

Keywords: Epitope, Vaccine, Allergy, Grass, Pollen, Cynodon dactylon, Poa pratensis, Phleum pratense

A novel multi epitope vaccine candidate Design against Brucellosis by in silico tools

Masoumeh Sadat Derakhsh[1], Zahra Zamanzadeh[1]

1. Department of Genetics, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani University, Isfahan, Iran

Background: Brucellosis, caused by Brucella species, is a highly contagious zoonotic infections worldwide with more than 500,000 human cases reported annually. There are still many clinical challenges in the diagnosis and treatment of brucellosis. Antibiotic therapy for human brucellosis is not always effective and there is no vaccine available for prevention of human brucellosis yet. Therefore, an effective vaccine should be designed to prevent Brucella infection. For this purpose, we applied the reverse vaccinology approach to design a multi-epitope vaccine containing Sod, Omp31, Tolc and L7/L12 antigens.

Methods: To identify probable conserved epitopes and choose epitopes based on allergenicity, toxicity, antigenicity, and molecular docking, computational analysis and immunoinformatics tools were employed for epitope prediction and screening. The selected epitope segments were then joined by the appropriate linkers in the following step. Finally, the new vaccine construct was attached to HBD3 as an adjuvant. The designed multi-epitope vaccine's secondary and third structures were predicted using in silico tools. Ultimately, molecular docking, molecular dynamics, and immunoinformatics evaluation were carried out to verify vaccine effectiveness. To guarantee the unique multi-epitope vaccine's expression yield in the target host, codon optimization and in silico cloning were carried out.

Results: The vaccine construct had 685 amino acids, a molecular weight of 69.6 kDa, was a soluble protein, and had antigenic and nonallergenic characteristics. The modeled protein has a stable structure that could interact with TLR4, TLR8 and TLR5 based on molecular docking studies, molecular dynamics simulations, as well as tertiary structure validation techniques. This new vaccine may thereby induce immunological responses in B and T cells and shield against B. suis, B. abortus, and B. melitensis infection.

Conclusion: According to immunoinformatics studies, this multi-epitope peptide vaccine may be expressed successfully and may be utilized to treat or prevent brucellosis.

Keywords: Brucellosis" Multi-epitope vaccine" in silico" vaccine design

Alteration of NEK7-NLRP3 inflammasome interactions by triple-H therapy in subarachnoid hemorrhage (SAH): Immune epitope mapping and in silico molecular dynamics study

Kiarash Saleki[1]

1. Shahid Beheshti University of Medical Sciences, Babol University of Medical Sciences

Background: NIMA-related kinase 7 (NEK7) is a direct regulator of the main inflammasome complex, NLR family pyrin domain containing 3 (NLRP3). Initially, through a forward genetic analysis found mutated NEK7 was linked to lower IL-1βexcretion, and further evidence indicated that NEK7 attaches to the leucine-rich repeat (LRR) region of NLRP3. NEK7 has been implicated in neurological disorders, such as neurodegenerative disorders, neuropsychiatric problems, and even neurosurgical conditions. NEK7 has been implicated in subarachnoid hemorrhage (SAH) which is sometimes treated by triple-H therapy, consisting of hemodilution, hypertension, and hypervolemia as well as nimodipine which prevents vascular spasm. We simulate the effect of these therapeutics and biophysical conditions on NEK7-NLRP3 interactions.

Methods: NEK7 and NLRP3 were retreived in PDB (rcsb.org). Docking with the Leucin-rich repeat of NLRP3 was performed by VinaAutoDock. A molecular dynamics study using nimodipine and docked NEK7-NLRP3 complex was performed in a simulation box in Groningen Machine for Chemical Simulations (GROMACS). Pressure and hemodynamics were changed to mimic SAH. Molecular Mechanics-Poisson Boltzman (MM-PBSA) analysis was performed for NEK7-NLRP3. Finally, immune epitope mapping by Immune epitopes database (IEDB) was carried out to provide clues for targeting NEK7-NLRP3 through immunotherapy.

Results: RMSD of the system stabilized within 10 ns of simulation. Contact residues were altered as a result of SAH-therapy-like simulation. Gyration analyses showed no denaturation of complex. RMSD analysis showed difference in fluctuation at binding site compared to normal blood condition. Immune epitope-mapping found both continuous and discontinuous B-cell epitopes and CTL/HTL epitopes. Protein linkers could be used to connect these epitopes and build novel immunotherapy candidates for SAH by targeting inflammasome pathways.

Conclusion: For the first time ever, we performed an analysis of SAH using MDS. These provide first clue for the role of routine therapies on inflammasome pathways and suggests immune targets to design immunotherapeutic candidates.

Keywords: Neuroimmunology, computational immunology, immune epitope-mapping, molecular dynamics simulation

Anticancer potential of Artemisinin and 1-phenyl-2, 4 pentadiyne on human gastric cancer AGS cells: an in-silico study

Elahe Aleebrahim-Dehkordi[1], Ali Jafari[2], Hamed Ghoshouni[3], Pooneh Koochaki[4], Neda Faal Hamedanchi[5], Farnosh Shadnoush[1], Amin Soltani[6], Mahmoud Rafieian-kopaei[7]

1. Shahrekord University of Medical Sciences, hahrekord, Iran.
2. Department of Nutrition, School of Health, Golestan University of Medical Sciences, Golestan, Iran.
3. Shahid Sadoughi University of Medical Sciences, Yazd, Yazd, Iran.
4. Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA.
5. Islamic Azad University, Tehran Medical Sciences branch, Faculty of Medicine, Tehran, Iran.
6. Cellular & Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.
7. Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Background: Malignant disorders of the apoptotic process can directly or indirectly lead to various diseases including cancer. Spread of cancer over the past years have led to the development of various treatment methods. The application of results from theoretical methods of computer software along with laboratory methods will accelerate our understanding of the interaction and mutual effect of molecules. This research was in-silico conducted with the aim of investigating the anticancer potential of artemisinin and 1-phenyl-2, 4 pentadiyne on human gastric cancer AGS cells.

Methods: The high-resolution X-ray diffraction of BCL-XL, BCL-XL/BAK, Bcl-2, p53, p300 and BimBH3mini (PDB IDs of 1G5J, 1BXL, 5VAU, 2K8F chain B, 2K8F chain A, and 4ZIF, respectively) structures were received from the Protein Data Bank (https://www.rcsb.org/pdb). Two major components of Artemisia absinthium extraction, including artemisinin and 1-phenyl-2,4 pentadiyne were downloaded from the ChEMBL database (https://www.ebi.ac.uk/chembl/). Discovery Studio software 2.5 (DS, Accerlys Inc, San Diego) were applied to delete water molecules and add hydrogen atoms in PDB structures at pH 7.

Results: Two selected ligands were docked in BCL-XL, BCL-XL/BAK, Bcl-2, p53, p300 and BimBH3mini. The global binding energy for artemisinin and 1-phenyl-2, 4 pentadiyne in were negative. The binding energy for artemisinin in BCL-XL/BAD, BCL-XL/BAK, Bcl-2, p53, p300 and BimBH3mini were obtained -30.39, -24.36, -37.97, -23.11, -34.56 and -31.40, respectively. Also, Atomic contact energy (ACE) in all the docked cases was a negative value. However, it seems like the hydrogen bindings did not generate in some complexes, including the artemisinin-BCL-XL/BAD, artemisinin-Bcl-2, artemisinin-P53, artemisinin-p300, and 1-phenyl-2, 4 pentadiyne-BimBH3mini.

Conclusion: molecular docking studies provided good information about the binding affinity of two compounds of the plant, artemisinin and 1-phenyl-2, 4-pentadiyne. The results of this study can be applied to conduct further studies on the medicinal effects of A. absinthium, the molecular processes affecting apoptosis and the involved gene changes.

Keywords: Anticancer, Artemisia, Molecular docking, Drug design, Gastric cancer

Bioinformatic analysis of Non-synonymous SNPs in human ADAM33 gene: The major frequent pattern of genetic association to asthma susceptibility

Milad Mohkam[1], Nasim Golkar[1], Seyed Hesamodin Nabavizadeh[1], Hossein Esmaeilzadeh[1], Aydin Berenjian[1], Ahmad Golami[1], Soheila Alyasin[1], Zahra Ghahramani[1]

1. Shiraz University of Medical Sciences, Shiraz, Iran.

Background: ADAM33 is a zinc-dependent metalloprotease belonging to the ADAM family that promotes the activation of Th2 cytokines and growth factors. Moreover, it plays a crucial role in determining lung functions throughout life for the developing fetus two months after gestation. In this regard, ADAM33 mutations may contribute to asthma risk. Consequently, identifying non-synonymous single-nucleotide polymorphisms (nsSNPs) associated with ADAM33 can be of great importance for asthma therapy.

Methods: In this study, we assessed the impact of nsSNPs in ADAM33 identified from the dbSNP database using more than 20 computational prediction tools. We also analyzed the structural impact of convergent detrimental alterations using molecular dynamics simulations.

Results: ADAM33 is a zinc-dependent metalloprotease belonging to the ADAM family that promotes the activation of Th2 cytokines and growth factors. Moreover, it plays a crucial role in determining lung functions throughout life for the developing fetus two months after gestation. In this regard, ADAM33 mutations may contribute to asthma risk. Consequently, identifying non-synonymous single-nucleotide polymorphisms (nsSNPs) associated with ADAM33 can be of great importance for asthma therapy.

Conclusion: ADAM33 is a zinc-dependent metalloprotease belonging to the ADAM family that promotes the activation of Th2 cytokines and growth factors. Moreover, it plays a crucial role in determining lung functions throughout life for the developing fetus two months after gestation. In this regard, ADAM33 mutations may contribute to asthma risk. Consequently, identifying non-synonymous single-nucleotide polymorphisms (nsSNPs) associated with ADAM33 can be of great importance for asthma therapy.

Keywords: ADAM33, Asthma, in silico analysis, Single-nucleotide polymorphism

Bioinformatic study reveals the difference in the expression level of ADAMDEC-1 in TCGA cancer panel

Shima Makaremi[1], Vahid Asghariazar[2], Elham Safarzadeh[3]

1. School of Medicine and Allied Medical Sciences, Ardabil University of Medical Sciences, Ardabil, Iran.
2. Cancer Immunology and Immunotherapy Research Center, Ardabil University of Medical Sciences, Ardabil, Iran. Deputy of Research and Technology, Ardabil University of Medical Sciences, Ardabil, Iran.
3. Department of Microbiology, Parasitology, and Immunology, Ardabil University of Medical Sciences, Ardabil, Iran.

Background: Cancer is the leading cause of death and a significant obstacle to raising life expectancy in every country in the world. According to the World Health Organization (WHO) estimates, cancer is considered as the first or second leading cause of death in 112 of 183 countries. Currently, metastasis is known as one of the main reasons for tumor progression. Mounting evidence indicates that tumor progression is frequently linked to the secretion of metalloproteinases that enable tissue invasion and intravasation by cancer cells via extracellular matrix (ECM) degradation. A Disintegrin and Metalloproteinase (ADAM) is a family of peptidase proteins that have diverse roles in tissue homeostasis and immunity. The biological function of ADAM-like DECysin-1 (ADAMDEC-1) still is unknown in diverse cancers. Hence, we evaluated the role of ADAMDEC-1 as a unique member of the ADAM family in The Cancer Genome Atlas (TCGA) cancers.

Methods: We performed a comprehensive bioinformatics analysis of expression of the ADAMDEC-1 across TCGA cancers (with tumor and normal samples) in various cancers. Besides, we conducted the correlation analysis to find genes related to ADAMDEC-1 expression.

Results: Our results demonstrated that the expression level of ADAMDEC1 is different (decreased or increased) in various tumor tissue compared with normal tissue depending on the cancer type. Moreover, TCGA analysis revealed 34 gene candidates that exhibited a positive correlation with ADAMDEC-1 across TCGA cancers.

Conclusion: Our study displayed that ADAMDEC1 can be considered as an influential biomarker in various cancer progression with diagnostic and prognostic advantages.

Keywords: Cancer, Tumor biomarkers, The Cancer Genome Atlas (TCGA), A disintegrin and metalloprotease like decysin (ADAMDEC1)

Characterization and 3D structural modeling of a specific single chain antibody against HER-2 antigen

Elham Nadimi[1], Seyed Mohammad Saleh Hadavi[2], Payman Bemani[3]

1. Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Shiraz, Iran.
2. Department of IT and computer Engineering Shiraz University of Technology, Shiraz Iran.
3. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Shiraz, Iran.

Background: ErbB2/HER2 is a member of the epidermal growth factor family of tyrosine kinase receptors which is involved in a variety of cases, such as gastric cancer, ovarian, colorectal, pancreatic and endometrial and breast cancer. It plays a critical rate in the differentiation, proliferation as well as migration of cancer cells. In the present study, we used homology modeling methods to predict 3D structure of anti-Her2 scFv.

Methods: The 3D structure model of the anti-Her2 scFv antibody was built by using the homology modeling-based method from the available crystal structure of single-chain antibodies. To assess the quality of a predicted model, ERRAT, and PROCHECK web servers were used. The ClusPRO program as first fully automated web-based molecular docking program was used to study antigen-antibody interaction. The three-dimensional structures of the human Her2 protein were retrieved from the Protein Data Bank. After docking, the best docking result with the lowest energy was selected and further analysis of the selected model was performed by the Chimera, the Chimera and PyMOL software, and PIC (Protein Interactions Calculator) webserver.

Results: The best 3D structure of anti-Her2 scFv protein indicated the selected model in comparison with other derived models has more stability and proper folding. The ERRAT result indicated the overall quality factor of model was 88.94. The stereochemical evaluation of backbone psi and Phi dihedral angles of the anti-Her2 scFv antibody revealed that 90.9 %, 6.5%, 1.6%, and 1.1% of residues were located in the most favored regions. Based on our docking results, 9 functional and key residues at the PCS participate in direct contact with residue from antigenic peptides on the Her2 protein.

Conclusion: Our data also showed tyrosine is the most frequent interacting residue forming an H bond.

Keywords: scFv, HER2 Antigen, docking, 3D modeling

Design of a multiepitope chimeric vaccine utilizing bacterial infections associated with Alzheimer's disease, cognitive impairment, and dementia via high-throughput Immunoinformatics approaches

Parsa Alijanizadeh[1], Kiarash Saleki[2], Nima Rezaei[3]

1. Student Research Committee, Babol University of Medical Sciences, Babol, Iran 2. USERN Office, Babol University of Medical Sciences, Babol, Iran.
2. Student Research Committee, Babol University of Medical Sciences, Babol, Iran 2. USERN Office, Babol University of Medical Sciences, Babol, Iran. 3. Department of e-Learning, Virtual School of Medical Education and Management, Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran.
3. Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran 5. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran 6. Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

Background: Infection with some critical organisms and viruses could be predisposing factors for Alzheimer's disease (AD), cognitive changes, and dementia. Infection by herpes simplex virus type 1, picornavirus, Borna disease virus, Chlamydia pneumonia, CMV, Hepatitis C virus (HCV), and Helicobacter pylori has been documented to play a part in the neuroimmunopathology of AD. Specific bacteria could also contribute to AD such as Porphyromonas gingivalis and Treponema denticola. These organisms boost Th1 immune responses. Here, we utilized the reverse vaccinology (RV) to direct immunity towards Th2 by producing an immunogenic multi-epitope vaccine against most common microbiota that contribute to AD.

Methods: Conserved domain atherogenic epitope from mentioned bacteria and viruses were identified. The sequence of chimeric-vaccine was prepared for the territory structure of the vaccine was created with MODELLER. The first 3D-model was refined via Ramachandran plot. The vaccine and TLR-4 were docked and used GROMACS for molecular dynamics (MD) simulation of vaccine and TLR4 complex. C-ImmSim server helped the immune stimulation of the chimeric-vaccine. It is necessary to predict of solubility, antigenicity, and allergenicity of a structure.

Results: Online bioinformatics servers were hired for predicting MHC-1, MHC-2, and CTL epitopes. These epitopes, TAT, adjuvant, and IL-10 inducer were connected by a linker. An enhanced ERRAT outcome of over 80 for the developed model confirmed it is stable. Hence, Ramachandran plot over 97% of the residues in the most permitted and favorable location. MD simulations displayed the docked vaccine-TLR4 were stable with many contact residues. RMSD stabilized and Molecular Mechanics Poisson-Boltzman Surface Area (MM-PBSA) energies were negative, indicating promising binding. Finally, immune response simulations showed favorable immune feedback biased towards Th2.

Conclusion: We created an immunogenic vaccine against AD and cognitive impairment and verified its favorable properties by Immunoinformatics. Future preclinical studies are warranted.

Keywords: Alzheimer's disease, cognitive impairment, chimeric vaccine, Immunoinformatics

Design of a new multi-epitope oral vaccine against brucellosis based on T and B cell epitopes by using bioinformatics methods

Morteza Abkar[1], Ali Torkian[1]

1. Shahid Ashrafi Isfahani University, Isfahan, Iran.

Background: The zoonotic illness brucellosis can be spread between domestic and wild animals and humans. Due to the disadvantages of commercial vaccines, there is an urgent need to design effective vaccines against the disease. In present study, bioinformatics tools were used to design a novel multi-epitope vaccine (MEV) from Brucella outer membrane protein 10 (Omp10), Omp16, Omp25, and Omp28.

Methods: Sequence retrieval and alignment, prediction of signal peptide, B and T cells epitope, assessment of antigenicity, toxicity, allergenicity and physicochemical aspects, molecular docking between T cell epitopes and MHC molecules, selection of adjuvant and protein linker for MEV construction, prediction of secondary and tertiary structures, evaluation of protein stability, molecular docking of MEV and TLRs (TLR2 and TLR4), molecular dynamics, immune simulation, mRNA structure prediction, codon optimization and cloning were performed.

Results: 21 T cell epitopes and 4 Linear B cell epitopes were identified after molecular docking between anticipated epitopes and MHC molecules. These epitopes are also highly antigenic and unlikely to result in allergy or toxicity. A 595 amino acid MEV construct was made using the expected epitopes along with the relevant linkers and the adjuvant unlipidated Omp19. The MEV has antigenicity, and solubility values of 0.8258 and 0.395, respectively. The MEV's Ramachandran diagram revealed that 97.1% of the residues located in favorite regions. Molecular docking was done to confirm the MEV TLR2 and TLR4's stability and affinities. The strong intermolecular interaction between the MEV and TLR receptors was further supported by molecular dynamics simulations. In addition, immune simulation results indicated that the MEV may induce secretion of IFN-γ, IL-12 and IgG by different immune cells.

Conclusion: Our findings demonstrated that this MEV construct has an excellent structure and acceptable properties, which could serve as a theoretical foundation for future laboratory investigations.

Keywords: Brucellosis, Multi-epitope vaccine, Molecular docking and dynamics, in silico tools

Designing an in-silico-based multi-peptide vaccine against the most common and allergenic weed pollen grains: Amaranthus retroflexus, Chenopodium album, Salsola kali

Mina Noroozbeygi[1], Mina Mohammad-rezaei[1], Mohammad-Ali Assarehzadegan[1]

1. Immunology Research Center, Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Pollen grain is one of the most common triggers of seasonal allergies. Worldwide, pollen from allergenic weeds represents an important allergen source and sensitized patients develop allergic symptoms during late summer until autumn. Amaranthus retroflexus, Chenopodium album and Salsola kali are among the most common weeds that cause respiratory allergies.

Methods: Ama r 1, Che a 1, and Sal k 1 are major allergens of the above-mentioned weeds respectively, used in this study to design a peptide allergy vaccine based on immunoinformatics methods. After retrieval of sequences of the main allergens, the following steps included: T cell epitopes selection, B cell epitopes selection, antigenicity, allergenicity, and toxicity evaluation, addition of linkers and adjuvant, secondary structure prediction, 3D structure modelling by GalaxyTBM, model validation by ProSA-web, ERRAT, RAMPAGE, and finally interaction of designed molecule with receptor was explored by SwarmDock.

Results: The results showed that 22VAKAPVS28 (for Ama r 1), 41CRIQFMTRISTIM53 (for Che a 1) and 6GGPEYRT12 (for Sal k 1) are the most potential predicted B cell epitopes, in addition to 8FLLVGALCVLSLDDV22 (for Ama r 1), 133ASDIRSANALGFMRK147 (for Che a 1) and 22SYVIVIKEPAEEFTT36 (for Sal k 1) as the most potential predicted T cell epitopes, which were interacted with MHC allelic protein HLA-DRB1*01:01, HLA-DRB1*09:01 and HLA-DRB1*08:02 respectively, with the lowest IC50 value.

Conclusion: Ultimately, the epitopes may be considered as potential peptides for peptide vaccine for weed pollen allergen after further experimental study.

Keywords: In silico, Epitope, Vaccine, Allergy, Weed, Pollen, Immunotherapy

Designing subunit vaccine to motivate immune system against the pathological form of hyperphosphorylation tau; Immunoinformatic and in silico study

Armin Ariaei[1], Fatemeh Ramezani[2]

1. Student Research Committee, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Physiology Research Center, Iran University of Medical Sciences, Tehran, Iran

Background: Alzheimer's disease requires urgent attention to conduct novel therapeutic and prevention approaches. Since tau is the most prominent biomarker of this disease and currently only two vaccines have entered the clinical trial stage, in this study, an active tau-based epitope was designed to stimulate B-cells to protect neurons from neurofibrillary tangles.

Methods: The sequence of the pathological form of the 3R isoform of tau was derived based on previous studies. The following epitope was grafted into a scaffold through the PyRosetta python module. The RMSD alternation of the grafted peptides was determined through Robetta and subsequently represented through the PyGnuplot python module. Moreover, the protective antigen prediction with a threshold of 0.4 was implemented through VaxiJen v2.0. Besides, the binding affinity of the receptor and the grafted peptide was calculated through Hex software. The cytotoxic T lymphocyte epitope prediction was performed through the NetCTL server. Finally, the stability of a human antibody in complex with grafted peptide was analyzed for 100ns with Coarse-grained Brownian molecular dynamics (MD).

Results: The predicted value for the protective antigen was calculated as 0.4854, while the NetCTL did not identify MHC ligands. Moreover, the average RMSD of the grafted peptide in the Robetta was 2.548 angstrom. The energy binding of the CBTAU-27.1 human antibody in complex with grafted peptide was -483.15 kcal/mol. Finally, the result of MD in the RMSD value in 100ns was 2.05 angstrom.

Conclusions: The predicted value for the VaxiJen v2.0 reported a probable antigen feature illustrating the potential for B cell stimulation in contrast to NetCTL results. The RMSD value calculated through Robetta demonstrated an unstable state due to the grafting, contrary to the molecular docking and dynamics results which depicted a stable posture. Eventually, it is suggested to perform an in vivo study to justify the in-silico results.

Keywords: Immunoinformatic, Vaccine, Alzheimer's disease, Tauopathy, Immune system

Diagnosis and prognosis of colorectal cancer based on artificial intelligence

Fatemeh Hajibabaie[1], Navid Abedpoor[2], Zahra Ahmadi[2]

1. Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
2. Islamic Azad University, Isfahan (Khorasgan), Isfahan, Iran.

Background: While several databases and evidence indicated that environmental agents, including gut microbiota, lifestyle, and metabolic disorders as potential contributors to gastrointestinal damage, colorectal cancer (CC) pathogenesis have not yet been fully comprehended. Hence, comprehending the pathomechanism and marking hub genes involved in colorectal cancer susceptibility can supply new perspicuity into prognosis, diagnosis, and therapeutic strategies.

Methods: Initially, non-coding RNA and related genes are collected from the GEO database. Subsequently, several parameters are determined to choose several thousand genes and non-coding RNAs using the R programming language. Finally, the program output is evaluated and analyzed by several ncRNA databases. In the end, a network between selected genes and non-coding RNAs is visualized by Cytoscape software.

Results: In-silico analysis designated a mRNAs-lncRNAs network involved in the CC. Bioinformatics analysis revealed IL17a, TLR2, IL10, STAT4, AKT1, NLRP3, MPO, ELANE, ARG1, AZU1, CD79a, RNASE3, and CTSG are essential hub genes in CC. Moreover, based on artificial intelligence, we found that several microRNAs and long-non-coding RNAs could regulate these hub genes and manage the CC progression.

Conclusion: Based on our data, the selected hub genes, microRNAs, and long-non-coding RNAs profiles could be considered diagnosis and prognosis biomarkers in the CC condition.

Keywords: Diagnosis, Prognosis, Colorectal Cancer, Hub genes

Downregulation of ADAMDEC1 Correlates with Tumor Progression and Metastasis in Colorectal Cancer: a Bioinformatic Study

Shima Makaremi[1], Vahid Asghariazar[2], Elham Safarzadeh[3]

1. School of Medicine and Allied Medical Sciences, Ardabil University of Medical Sciences, Ardabil, Iran.
2. Immunology Research Center, Ardabil University of Medical Sciences, Ardabil, Iran.Deputy of Research and Technology, Ardabil University of Medical Sciences, Ardabil, Iran.
3. Deputy of Research and Technology, Ardabil University of Medical Sciences, Ardabil, Iran.Department of Microbiology, Parasitology, and Immunology, Ardabil University of Medical Sciences, Ardabil, Iran.

Background: Colorectal cancer (CRC) as a heterogeneous disease is the third most common cancer with a high incidence rate worldwide. Despite improvements in patient outcomes and advancements in therapies such as surgery, chemotherapy, and targeted medicines CRC remains the largest cause of mortality. Therefore, finding distinctive and common characteristics in their molecular and biological processes might provide new insight into the creation and identification of diagnostic and therapeutic biomarkers. The release of metalloproteinases, which facilitate tissue invasion and intravasation by cancer cells through extracellular matrix (ECM) breakdown, is usually associated with tumor growth. A disintegrin and metalloprotease like decysin (ADAMDEC1) as a member of a disintegrin and metalloprotease (ADAMs) family play diverse roles in tissue homeostasis and immunity. Here, we aimed to explore the function of ADAMDEC1 in CRC.

Methods: Clinical information and mRNA expression levels of ADAMDEC1 metalloprotease in patients with CRC were obtained from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov) based on the following selection criteria including basic clinical information of stage, gender, sample types (the normal and primary tumor), histological subtypes and nodal metastasis status. All requirements were conducted on a large sample size (>250). Datasets were then compared to obtain the significant p-value.

Results: Our results indicate that the expression level of ADAMDEC1 is down-regulated in CRC compared with normal tissue. This decrease was more considerable in stage 4 and N1 metastasis status of the disease ($p< 0.001$). In the case of histological subtypes, the expression of ADAMDEC1 in adenocarcinoma shows more reduction compared with mucinous adenocarcinoma ($p< 0.001$).

Conclusion: Our study revealed that ADAMDEC1 can be considered an influential factor in CRC progress with diagnostic and prognostic values.

Keywords: Tumor biomarkers" Colorectal cancer (CRC)" The Cancer Genome Atlas (TCGA)" A disintegrin and metalloprotease like decysin (ADAMDEC1)

Expression Changes of TP53, HRAS, RELA, RAC1, and CDC42 as Potential Therapeutic Targets in Multiple Sclerosis

Maryam Rahnama[1], Elham Talebi[2]

1. Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran
2. Department of Genetics, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

Background: The myelin sheathing of the brain and spinal neurons is harmed in Multiple Sclerosis (MS), a prevalent autoimmune nervous system disorder. The immune cells' attack on the nerves is one of the primary causes of the disease. Since there is no effective treatment for MS, this work sought to uncover MS-related gene expression alterations in T-cells, in order to identify candidate biomolecules with further diagnostic and therapeutic potentials.

Methods: GSE43591 dataset, including the peripheral blood of 20 MS and healthy individuals, was obtained. Differentially expressed genes (DEGs, FDR < 0.05, |FC| > 1) were screened using the Limma package in R. Pathways associated with DEGs were identified through the Enrichr database. Then, the protein-protein interaction (PPI) network was constructed using STRING and further analyzed through Cytoscape software. The centralities (degree, closeness, betweenness, and eigenvector) were calculated to explore the hub genes. In addition, subnetwork analysis was also performed to identify significant modules.

Results: The findings indicated that 1333 genes were significantly altered in T-cells of MS patients compared to controls, of which 301 genes were up and 1039 genes were down-regulated. DEGs were found to be involved in pathways such as inflammation, apoptosis, PD-L1, and ubiquitin contributed to MS pathophysiology. Furthermore, several genes such as TP53, HRAS, RELA, RAC1, and CDC42 were determined as hub genes based on PPI network analysis. Moreover, 5 sub-networks were identified whose related genes were involved in pathways including cell proliferation, apoptosis, inflammation, ubiquitin, and interferon-gamma.

Conclusion: Changes in the expression of genes such as TP53, HRAS, RELA, RAC1, and CDC42 might be related to MS pathology. It is suggested that hub genes in addition to critical dysregulated pathways may serve as potential therapeutic and diagnostic targets in MS. Further experimental studies in large sample sizes are indeed required to validate these candidate biomarkers.

Keywords: Multiple Sclerosis, Immune system, Protein-protein interaction network, Enrichment

Identification of conserved epitopes for new Monkeypox vaccine platforms: A novel immunoinformatic and machine learning approach

Mohammad Izadi[1], Fatemeh Mirzaei[1], Amirhossein Mazare[1], Fatemeh Zahra Ehsani[1], Masoumeh Basirat[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: In 2022, Monkeypox outbreaks were reported in several non-endemic regions, and the accelerated evolution of the virus has raised concerns about the efficacy of the smallpox vaccine in preventing Monkeypox. To address this, we developed a method to identify conserved epitopes for new vaccine platforms based on Monkeypox sequences.

Methods: We obtained Monkeypox sequences from the National Center for Biotechnology Information Virus database and used Vaxign2 and Cello2go to determine target proteins for epitope prediction. We predicted MHC-I epitopes using NetCTLv1.2, MHC-II epitopes with a length of 15 using the Peptide binding to MHC class II molecules tool, and linear B cell epitopes using Bepipred. We assessed epitope toxicity, allergenicity, and antigenicity using Toxinpred, Allertop, and Vaxijen2, respectively. We also used NextClade to calculate the number of mutations and Unsupervised Nearest Neighbor (UNN) machine learning algorithm to calculate the conservancy score of epitopes.

Results: Our analysis identified six proteins for epitope prediction and resulted in 136 MHC-I epitopes, 35 MHC-II epitopes, and 10 Linear B cell epitopes. After calculating the number of mutations and sites of mutations for each epitope, we discovered 47 MHC-I, 5 MHC-II, and 10 Linear B cell conserved epitopes using the UNN algorithm. Our results indicate the existence of 2337 SNPs in Monkeypox sequences until October 2022, suggesting that the virus is undergoing accelerated evolution.

Conclusion: Our study highlights the importance of developing new vaccine platforms based on conserved epitopes to prevent viruses with high mutation rates and accelerated evolution, such as Monkeypox. Our method can be used to identify conserved epitopes for other rapidly evolving viruses, and our findings provide a starting point for developing more effective Monkeypox vaccines.

Keywords: Conserved epitopes, Monkeypox, Immunoinformatic, and Vaccine design

Identification of T cell epitopes targeting a biofilm formation protein of Staphylococcus aureus: An immunoinformatics approach

Parisa Khodabandeh[1]

1. Isfahan Endocrine and Metabolism Research center, Isfahan, Iran.

Background: S. aureus is the pathogen most frequently isolated from diabetes foot ulcer. These days, antibiotic-resident S. aureus is an active debate in science and clinical world. One of the pathways of biofilm formation of S. aureus is performed by the polysaccharide intercellular adhesion (PIA) encoded by the ica operon. Expression of ica A have been frequently reported in S. aureus isolates from diabetes foot ulcer. In current study, we harness immunoinformatics tools to find out potential T cell epitopes for this protein.

Methods: We used IEDB MHC-II Binding Predictions tools and NetMHCIIpan-3.2 to identify T cell epitopes of the protein encoded by ica A locus. Common epitopes were selected then antigenicity, toxicity and allergenicity, population coverage and conservancy were evaluated. The final epitopes were blasted with human proteome to evaluate the risk of autoimmunity if these epitopes use in a multi-epitope vaccine.

Results: Among epitopes that represent by IEDB MHC-II Binding Predictions tools and NetMHCIIpan-3.2 “DQDAPYYMIENFKHDPKL”, “NAAVVLVAFPKALKRKKG” and “IVGSIYFYFTREIRYSL” displayed the highest affinity for MHCII and were recognized by at least two HLA-II high frequent alleles. Although “DQDAPYYMIENFKHDPKL” revealed high level of population coverage, it was predicted as probable allergen by Allergen FP server. Other epitopes were predicted as non-allergen and non-toxin epitopes.

Conclusion: The core region of “NAAVVLVAFPKALKRKKG” have strong homology with several human proteins.

Keywords: S. aureus, Staphylococcus, aureus, T-cell epitopes, bioinformatics, Computational approaches, insilico, Vaccine design, ica A

Immunoinformatics-based design of a novel chimeric subunit vaccine candidate for immune response reinforcement against Salmonella Typhi.

Mohsen Bidar Ajerloo[1], Abbas Hajizade[1], Shahram Nazarian[1]

1. Biology Research Center, Faculty of the Basic Sciences, Imam Hossein University, Tehran, Iran

Background: Salmonella enterica serovar Typhi (S. Typhi), a causative agent of typhoid fever, is a major health problem that causes significant morbidity and mortality all over the world. The currently available typhoid vaccines have poor immunogenicity and long-term efficacy. Due to these limitations and the emergence of multidrug-resistant strains, the development of a potent vaccine is urgently needed. The present study aims to design a chimeric subunit vaccine against S. Typhi.

Methods: Following a broad literature review, three important immunogens were selected: FliC, SteD, and STIV. The amino acid sequences of these proteins were obtained from Uniprot. The proteins were linked together via a rigid linker. Secondary and tertiary structures of the protein were predicted and validated using appropriate tools. The physicochemical properties of the protein, including its molecular weight, pI, instability index, etc., were predicted through ProtParam. The protein's antigenicity, allergenicity, and toxicity were determined using Vaxigen, AlgPred, and ToxinPred, respectively. Linear and conformational epitopes were also predicted using related programs. The human immune responses were simulated using the C-IMMSIM tool. The interaction of the vaccine candidate with immune system molecules was analyzed by molecular docking.

Results: The protein structure analysis showed that the three proteins had been successfully separated in the final chimeric protein and maintained their native secondary and tertiary structures. Physiochemical analysis showed that this 60 kDa protein has proper solubility and stability. The conformational and linear epitopes were preserved in the final structure. The immune response simulation showed that the protein could evoke human immune responses efficiently. The molecular docking showed that the protein could properly interact with immune system molecules.

Conclusion: According to the results of bioinformatics and immunoinformatics, the designed chimeric protein could be used as an immunogen to confer immunity against S. Typhi.

Keywords: Vaccine, S. Typhi, immunoinformatic, chimeric protein

In silico analysis of a multi-epitope-based vaccine designed by conserved epitopes of hemagglutinin protein from H1N1 and H3N2 influenza viruse

Farnaz Moghadamdizajherik[1], Reza mansouri[1], Majid Tabianian[2], Morteza Taghizadeh[2]

1. Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Research and Development Department, Razi Vaccine and Serum Research Institute, Agricultural Education and Extension Research Organization, Karaj

Background: Recently, there have been increasing interests in developing a new multi-epitope influenza vaccine which are based on the selection of targeting epitopes from pathogenic strains. Here, we aimed to design a novel multi-epitope influenza recombinant protein based on the highly conserved epitopes of hemagglutinin (HA) protein epitope from H1N1 and H3N2 strains.

Methods: After retrieving of influenza HA protein sequences and multiple alignments, the extracted sequences were analyzed by some bioinformatics online servers and datasets to select the highest potential T-cell and B-cell epitopes. After evaluation of physicochemical properties and solubility of the selected epitope, the Secondary structure of the recombinant vaccine was predicted. Codon optimization was performed following reverse translation of the sequence.

Results: The molecular weight (MW) of the designed recombinant protein is 28790.09 g/mol consists of 271 amino acids with good solubility score. Theoretical isoelectric point (pI) is 5.22 and the extinction coefficient was predicted as 33710 M-1cm-1. The coding index (CAI) was 0.96 and GC percentage was calculated as 57.88%.

Conclusion: The proposed multi-epitope recombinant protein has acceptable physicochemical and immunological properties and could be candidate for prokaryotic expression and immunological studies.

Keywords: "Influenza Vaccine" "Hemagglutinin" "recombinant protein"

Integrated Bioinformatics Analysis Reveals Key Candidate Genes and MicroRNAs in Hepatocellular Carcinoma

Ata moghimi[1], Farhad Jadidi-Niaragh[2], Nasrin Bani Hosseinian[1]

1. Department of Medical Laboratory Science, Tabriz University of Medical Sciences, Tabriz, Iran.
2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Hepatocellular carcinoma (HCC) is a type of primary liver tumor with poor prognosis and high mortality, and its molecular mechanism remains elusive. This study aimed to use bioinformatics technology to identify differentially expressed genes (DEGs) in HCC pathogenesis, hoping to identify novel biomarkers or potential therapeutic targets for HCC research and find MicroRNAs to target the hub genes.

Methods: The bioinformatics analysis of our research mostly involved the following two datasets: Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA). First, we screened DEGs based on the TAC (*P*: 0.01). Through the Cytoscape database, we selected our 10 hub genes with (degree ≥ 99, betweenness centrality ≥ 0.022, closeness centrality ≥ 0.104), and through the miRDB database, we selected suitable MicroRNAs with the highest targeted score.

Results: We ended up with 2530 DEGs, including 805 upregulated and 1725 downregulated DEGs. From the PPI, we filtered out ten hub genes, and these genes were significantly upregulated in HCC samples. Survival analysis showed high-level gene expression of ASPM, RRM2, CCNB1, KIF14, MKI67, SHCBP1, CENPF, ANLN, HMMR, and EZH 2, which were associated with the poor overall survival of HCC patients. For each gene, we suggested specific microRNA. In the order of the above genes, we found various microRNAs including has-miR-10522-5p, has-miR-4666a-3p, has-miR-548n, has-miR-3646, has-miR-7154-3p, has-miR-3163, has-miR-338-5p, has-miR-6807-3p,-548au-3p, and has-miR-101-3p.

Conclusion: The present study screened out the key genes and pathways related to HCC pathogenesis, which could provide new insight for the future molecularly targeted therapy and prognosis evaluation of HCC.

Keywords: HCC, Bioinformatics, hub gene, miRNA

Introducing an allergy vaccine designed by immunoinformatics methods for the most allergenic tree pollen in Iran (Platanus orientalis, Fraxinus excelsior, Betula pendula

Mina Mohammad-rezaei[1], Mina Noroozbeygi[1], Mohammad-Ali Assarehzadegan[1]

1. Immunology Research Center, Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Allergic diseases are one of the most common health problems worldwide, with more than 25% of people affected by type I hypersensitivity reactions. Allergic reactions have different causes and allergens from pollen grains are considered as a potential source of hypersensitivity reaction.

Methods: Platanus orientalis, Fraxinus excelsior and Betula pendula are among the most allergenic trees. Pla or 3, Fra e 1, and Bet v 1 are major allergens of Platanus orientalis, Fraxinus excelsior and Betula pendula respectively, which are used in this study using immunoinformatics methods to design a peptide allergy vaccine. The steps after finding the main allergens include sequences retrieval, T cell epitopes selection by IEDB and NetMHC, B cell epitopes selection by IEDB and BCpred, evaluating antigenicity, allergenicity and toxicity, addition of linkers and adjuvant, secondary structure prediction, 3D structure modelling by GalaxyTBM, validation the model by ProSA-web, ERRAT, RAMPAGE, Exploring interaction of designed molecule with receptor by SwarmDock.

Results: The results of our study showed that 23HAEAAITC30 (for Pla o 3), 5PQPPVS10 (for Fra e 1) and 41SVENIEGNGGPGTIKKI57 (for Bet v 1) are the most potential predicted B cell epitopes, while 37LTPCLTYLRSGGAVA51 (for Pla o 3), 104LKFILNTVNGTTRTI118 (for Fra e 1) and 17ARLFKAFILDGDNLF31 (for Bet v 1) are the most potential predicted T cell epitopes interacted with MHC allelic protein HLA-DRB1*01:01, HLA-DRB3*02:02 and HLA-DRB3*01:01 respectively, with the lowest IC50 value.

Conclusion: The designed vaccine could be used as a potential vaccine. However, the results need to be confirmed by immunological tests.

Keywords: in silico, Epitope, Vaccine, Allergy, Tree, Pollen, Immunotherapy

Lon Protease of Escherichia Coli as a Possible Therapeutic Candidate against Bladder Cancer Using Bioinformatics Analysis

Amirreza Mehmandar Oskuie[1], Forouzan Karimi[1], Masoud Tohidfar[2]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Department of Plant Biotechnology, Faculty of Life Science and Biotechnology, Shahid Beheshti University, Tehran, Iran.

Background: The use of bacteria or their secretory products has always been one of the possible therapeutic options for the treatment of various diseases, including cancer. In a previous study by Butler et al., has been shown that a specific strain of Escherichia coli produces Lon protease and degrades c-MYC of bladder cancer cells, as well as downregulates its related genes and finally, it prevents the growth of cancer cells. However, the effect of Lon protease on immune-related genes remains unexplored.

Methods: In this study, first, we analyzed data about the treatment of recombinant Lon protease (rLon) on bladder cancer cells which is available in the GEO dataset in NCBI through bioinformatics tools and R software. Following that, we identified differentially expressed genes (DEGs) and performed gene ontology (GO) and pathway analysis in Enrichr (https://maayanlab.cloud/Enrichr//). Furthermore, we used the STRING website (https://string-db.org) and the Cytoscape software to build a protein-protein interaction (PPI) network.

Results: We found that rLon of E. coli can reduce the inflammatory/immune response pathways and the expression of certain cytokines and chemokines or their receptors such as CCL2, CX3CL1, and IL-2R which are related to the immune system. Surprisingly, this compound has the remarkable ability to downregulate the TP53 as a tumor suppressor gene as well as other genes linked to tumor growth such as MMP-9.

Conclusion: Our study suggested that based on in silico information, rLon of E. coli has the potential to decrease tumor progression while also reducing immunological migration and response. However, in vitro and in vivo studies are further needed to validate these findings.

Keywords: Bladder cancer, Lon protease, Cytokines, Bioinformatics

Microbial view of IBD and molecular mimicry: the two sides of the coin

Mahsa Alem[1], Seyyed Meysam Abtahi Froushani[1], Nasim Hajighahramani[2]

1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Department of Pharmaceutical Biotechnology, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran

Background: The digestive system is impacted by inflammatory bowel disease (IBD), which is mostly brought on by dysbiosis of the gut microbiome. Information on the pathogenesis of inflammatory bowel disease is scarce. This prompted us to investigate whether any bacterial protein might be connected to the immunopathogenesis of inflammatory bowel disease.

Methods: A key strategy for locating the indicated target would be the bioinformatic analysis of host-microbe interactions. The proteome of the microorganisms described in the literature review related to IBD was examined for protein sequences with identities more than 35 percent using the NCBI's basic local alignment search tool for protein (BLASTP) and T-coffee expresso. Using Multiple Sequence Alignment from Protein Data Bank (PDB), the phylogenetic tree and degree of similarity between the protein sequences of bacteria and the human proteome were established.

Results: Microorganisms linked to IBD in this study included Campylobacter, Clostridium, Escherichia coli, Klebsiella, Listeria, Mycobacterium, Salmonella, Shigella, and Yersinia. This research discovered signs of molecular mimicry (molecular-level similarity between microbial antigens and host proteins). The 60, 70, and 90 kilo Dalton heat shock proteins (HSP) of microorganisms and humans were discovered as potential molecular targets because to their great conservation in evolution, which was followed by autoreactive T lymphocytes against heat shock proteins in humans. This point along with a dysbiotic gut microbiome may be a potential etiology in IBD. Finally, using the Immune Epitope Data Base (IEDB) tool, cytotoxic T lymphocyte and helper T lymphocyte epitopes with high homology to 60 (3 targeted epitopes), 70 (6 targeted epitopes), and 90 (4 targeted epitope) kilo Dalton heat shock proteins were extracted (even more than 90 percent in this research).

Conclusion: Last but not least, this study supports the idea that bacteria and the human proteome likely share many cross-reactive T cell epitopes by using an in silico immunoinformatic approach.

Keywords: Inflammatory Bowel Disease (IBD), Molecular mimicry, Heat-shock proteins (HSPs), Immunoinformatic

Molecular Docking new compounds against main proteins of SARS-COV-2

Rezvane Zoobinparan[1], Faezeh Ghasemi[1]

1. Blood Transfusion for Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

Background: A new virus called SARS-COV-2 originated in China and then caused a new pandemic all around the world from December 2019 until now. Several vaccines have been designed for this infection. However, we know that not all the vaccines are safe, and not all the people can be vaccinated, so it is critical to find more effective drugs for protecting people against this virus.

Methods: We accomplished structure-based virtual screening (SBVS) of a library that was built from bioinformatic databases against two target proteins of SARS-CoV-2, including main protease or MPro, S-protein or spike. We performed a virtual screening study using Docking software's.

Results: Our outcomes indicate that 12 top-ranked drugs with lower energies bind two target proteins of SARS-COV-2 and are possible inhibitors of these targeted proteins. Therefore, we suggest that these compounds are probably effective against this virus and have the potential to generate new efficient antiviral drugs.

Conclusion: we should develop our knowledge of coronavirus structure, such as different virus target proteins, for detection of new compounds for COVID-19. In addition, Clinical trial and experimental assays are essential to validate the actual activity of this compounds against this infection.

Keywords: COVID-19, Molecular docking, Virtual screening, Spike protein

Predicting the functional consequences of non-synonymous SNPs in Interleukin-35

Samaneh Tabaee[1], Samira Tabaei[2], Hassan Dana[3]

1. Noncommunicable Disease Research Center, Neyshabur University of Medical Science, Neyshabur, Iran
2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Faculty of Medicine, Neyshabur University of Medical Science, Neyshabur, Iran

Background: Interleukin 35 (IL-35) is capable of potently inducing IL-35- producing induced regulatory T cells (iTr35) to limit inflammatory responses. This study systematically collected non-synonymous SNPs information for the IL-35 gene from SNP databases and literature and screened for nsSNPs with high risks of pathogenicity in coronary heart disease (CHD).

Methods: This study systematically collected non-synonymous SNPs information for the interleukin-35 gene from SNP databases and literature and screened for nsSNPs with high risks of pathogenicity. The sequence homology-based genetic analysis of a set of 172 coding nSNPs associated with 10 rsIDs using Sorting Intolerant from Tolerant (SIFT) and Protein Variation Effect Analyzer (PROVEAN) identified 9 nsSNPs to be putatively damaging/deleterious in at least one of the two tools used. The structure homology-based Polymorphism Phenotyping (PolyPhen-2) analysis predicted 7 of 172 nsSNPs to be damaging.

Results: Based on stability analysis, RSMD and docking analysis, conservation of amino acid residues, structural superimposition, the possible structural-functional relationship was ascertained for high-confidence nsSNPs. The IL35 deregulation has also appeared to be an important prognostic marker for detection of patients with CHD.

Conclusion: This study, showed the effects of amino acid substitutions on IL35 protein structure, function and disease association.

Keywords: Coronary heart disease, Polymorphism, IL-35, Pathogenicity prediction, Bioinformatics

Production of a stable cell line expressing HER2 receptors devoid of dimerization domain: a platform for subtractive phage display

Ahmad Najafi[1], Mohsen Tehrani[1], Alireza Rafiei[1], Gholamreza Hashemitabar[2], Milad Lagzian[3], Armin Akbar[1], Reza Valadan[1]

1. Mazandaran University of Medical Sciences, Sari, Iran.
2. Ferdowsi University of Medical Sciences, Mashhad, Iran.
3. University of of Sistan and Baluchestan, Zahedan, Iran

Background: Dimer-dependent phosphorylation of HER2 receptor is a key event for the signal transduction of HER family of receptors which correlates with invasion and metastasis of several tumors. Subtractive phage display allows highly specific selection for antibody fragments. Selection of conformational antibodies against dimerization domain of HER2 requires HER2Δ249-265 and HER2 expressing cells to be used for negative and positive selection during bio panning procedures.

Methods: Bioinformatics analysis was used to selection of suitable sequence for deletion in HER2 receptor and then, the stability of the receptor on the cell surface through serial passages of VERO cells was confirmed using immunoblotting and immunofluorescence assays.

Results: Bioinformatics analysis revealed determine whether dimerization domain loop (235-275) of HER2 receptor is a suitable region for antibody production for several immunological properties, such as flexibility, antigenicity, and hydrophobicity. Analysis of structure-based antibody prediction indicated the 252-263 amino acid region as an accessible region for antibodies. After deletion of different sites of dimerization domain, stability evaluation of the HER2 receptor revealed that the 249-265 amino acid region is the best to be region to be deleted. Molecular Dynamics analysis indicated that both wild-type and mutant forms were fully consistent with the experimental data and showed that the mutant receptor was as stable as the wild-type. Immunoblotting and immunofluorescence assays showed that this receptor was stable on the cell surface through serial passages.

Conclusion: We established VERO stable cell lines expressing high levels of HER2Δ249-265. This cell line, not only provided platforms for phage display-based methods but also could be widely used as in vitro models to study different properties of HER receptors, including function, genetics, expression, and regulation, as well as cellular responses to newly developed therapeutics.

Keywords: Stable cell line, HER2, Phage display

T2A Linker-based in-silico modeling of a live non-pathogenic Leishmania tarentolae vaccine expressing two promising candidates from sandfly vector

Mahya Sadat Lajevardi[1], Tahereh Taheri[1], Elham Gholami[1], Negar Seyed[1], Sima Rafati[1]

1. Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran

Background: Vaccines against complex organisms such as Leishmania parasites will obviously benefit from multi-protein rather than single-protein-based formulations. This necessitates a preliminary evaluation approach to select the most favored combination of the proteins in the vaccine structure.

Methods: In this study, protein homology modeling along with mRNA analysis is introduced as a handy approach to rationally design a multi-protein vaccine using new virus-derived linkers. PpSP15 and PsSP9, two immunogenic sandfly-vector derived promising vaccine candidates, were fused together with or without linker (T2A peptide) to design an effective live vaccine against cutaneous leishmaniasis. All four possible combinations were structurally analyzed at both mRNA and protein levels. At the RNA level, first the full-length mRNA derived from the Leishmania tarentolae expression cassette was estimated. Then, the secondary structures of mRNAs resulting from different protein combinations were predicted (RNAfold). At the protein level, the basic 3D structure of all the combinations was modeled (I-TASSER), refined (GalaxyRefine) and validated (ProSA-web, PROCHECK, and QMEAN).

Results: Verified 3D models were then superimposed over the original single proteins (Chimera) to find the structural similarity. All the combinations were also further analyzed for junctional epitopes by immunoinformatics. At the mRNA level, only the mRNA of PpSP15-T2A-PsSP9 combination was most favored based on the energy related criteria. At the protein level, all the models derived from different combinations were structurally valid, however the local quality for each amino acid position estimated by QMEAN indicated the higher stability in PpSp15-T2A-PsSP9 combination. Moreover, the PpSp15-T2A-PsSP9 fusion was well superimposed over two original proteins with lowest RMSDs. No immunogenic junctional peptides were also detected for this structure.

Conclusion: The high expression level of the final selected structure evaluated in Leishmania tarentolae confirmed that the mRNA analysis as a criteria along with homology modeling approach can further improve the multi-protein vaccine design for any organism.

Keywords: Vaccine design, Structural analysis, Immunoinformatics, Leishmaniasis, Salivary proteins

The prediction of functional, stability, and pathogenicity of SARS-CoV-2 associated with N501Y and A570D spike mutations.

Mostafa Manian[1]

1. Iran University of Medical Science, Tehran, Iran.

Background: Mutations of the SARS-CoV-2 spike protein are common and have frequently been reported. Because of their possible impact on the pathogenesis of SARS-CoV-2, vaccine strategies, and the application of antibody-based therapies, it is critically important that their biological and molecular implications are investigated.

Methods: Here, we have used bioinformatics tools to examine the functional, potential pathogenicity, and stability changes associated with the N501Y and A570D mutations of the spike protein.

Results: We have also compared the native and mutated structures of SARS-CoV-2 spike protein to evaluate their binding energy with Angiotensin-converting enzyme 2 (ACE2) using protein-protein docking.

Conclusion: Further studies about N501Y and A570D mutations are still required.

Keywords: COVID-19, SARS-CoV-2, Protein-Protein Docking, Mutations, Spike protein, Bioinformatics.

The protection quest is a primary key to sharing the neutralizing antibody response to cover against all emerging VOCs based on BIV1-CovIran studies

Maryam Shafaati[1], Kowsar Bagherzdeh[2], Asghar Abdoli[3]

1. Department of Microbiology, Faculty Science, Jahrom Branch, Islamic Azad University, Jahrom, Iran
2. Eye Research Center, the Five Senses Health Institute, Rassoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
3. Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran

Background: Over time, the antigenic evolution of emerging variants of (SARS-CoV-2 has demanded the potential protective vaccines. Although several favorable efficacy results have been reported for current COVID-19 vaccines, the vaccines developed to date have not been able to meet global demand. Administration of additional doses of current vaccines based on the wild type spike protein may boost, but their effectiveness has dwindled for patients with more recent variants.

Methods: The neutralization activity of post-wild type strain-based vaccination and a structural simulation in-silico based on the interactions of the RBD-hACE2 as the key to initiating infection among the VOCs of SARS-CoV-2.

Results: The obtained data display that wild type sera showed markedly more reduction in Delta and Omicron, suggesting that the Wuhan-based vaccines may be more susceptible to breakthrough and new VOCs. According to the molecular dynamic simulations-based bioinformatic analysis of the interactions between wild type spike-RBD, as well as Alpha, Beta, Delta, and Omicron VOCs with hACE2 show that mutations of Omicron result in a significant change in the variant charge distribution throughout the binding interface that consequently alters the critical interface electrostatic potential in comparison to other variants.

Conclusion: It is speculated that a new generation of COVID-19 vaccines that provide cross-protective immunity against emerging SARS-CoV-2 variants is one of the future strategies in vaccine science. Updating COVID-19 vaccines will open the way for R&D in future epidemiology, because future pandemics are more likely. The more prepared we are, the more lives we will save.

Keywords: SARS-CoV-2 Vaccines, Variants of concern, Neutralizing antibody, Molecular dynamic simulations

Transcriptome-based and Regulatory Network Analysis Reveals Potential Biomarkers in Severe Asthma

Maryam Rahnama[1], Mona Ghourchian[2]

1. Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran
2. Department of Medical Biotechnology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran

Background: Severe asthma is a heterogeneous disease with uncontrolled symptoms, frequent exacerbations, and airway obstruction that are responsible for more than 60% of costs, and affect approximately 10% of patients. Treatment with inhaled corticosteroids does not relieve symptoms and asthma attacks return. Thus, understanding the molecular mechanisms and investigating novel treatments are of crucial importance. Transcriptome profiling in severe asthma has provided insights to identify candidate biomarkers associated with clinical symptoms.

Methods: The microarray dataset GSE143303, including endobronchial biopsies of 47 cases with severe asthma, and healthy controls was downloaded. Gene expression analysis was performed via R environment (FDR<0.05, |FC| > 1.2). Then, the STRING was used to construct and functionally analyze the protein-protein interaction (PPI) network. Moreover, hub genes were identified through the Cytoscape software (v.3.9.1) Furthermore, key miRNAs and transcription factors were explored via gene regulatory network construction.

Results: A total of 996 unique differentially expressed genes (DEGs) were identified. Gene ontology enrichment analysis revealed that DEGs were mainly involved in immune system regulation (FDR=1.12E-12), organic substance (FDR=4.01E-12), nitrogen compound (FDR=1.35E-11), and other cellular metabolic processes. They also exert their functions mostly via binding to heterocyclic compounds, enzymes, and mRNA, acting as activity regulators. Moreover, mTOR and PI3K-Akt signaling pathways, cellular responses to stress, and mitochondrial dysfunction were among the significantly dysregulated pathways. Furthermore, EP300, HSPA8, POLR2A, RHOA, and CCND1 genes were identified as hubs all with experimental evidence indicating their involvement in the pathophysiology of Asthma. GRN analysis also represented the let-7 family and miR-19, in addition to NF-κB and STAT, as critical regulators of immune processes reported to be associated with severe Asthma.

Conclusion: results of this study provided promising clues to the underlying mechanisms of severe asthma, and suggested biomolecules that may serve as candidate biomarkers in Asthma, need to be confirmed through further investigations.

Keywords: Asthma, Biomarker, Protein-protein interaction network, miRNA

Whole Blood Transcriptome Analysis in Autoimmune Patients

Navid Kashani[1], Fatemeh EmamiPari[2], Mostafa Khedrinia[3], Amir Sabbaghian[4]

1. Master of Cell and Molecular, Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Gorgan branch, Gorgan, Iran
2. Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
3. Master of General Biochemistry, Department of Biology, Faculty of Science, Golestan University, Gorgan, Iran
4. Student Research Committee, Department of Molecular Medicine, School of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran.

Background: Autoimmune diseases result from a disorder in the immune system caused by complex environmental and genetic factors and interactions. Different gene expression in patients compared to the control group has been shown in several studies. These factors affect the severity, type of disease, and how it responds to treatment. Therefore, the present study was conducted to investigate new biomarkers that may have use in therapeutic or prognostic agents.

Methods: The RNA-sequencing dataset, PRJNA258216 (Accession: GSE60424, ID: 200060424), was retrieved from the Gene Expression Omnibus (GEO). The datasets included a total of 134 blood samples, of which 21 were ALS, 28 were healthy controls, 20 were sepsis, 19 were MS without any treatment, 28 were MS post-treatment, and 26 patients had type 1 diabetes. MS post-treatment and type 1 diabetes were discarded due to the low quality of their reads and because of being treated with other factors. For the preprocessing step, FASTQC was used to control the quality of reads. TRIMGALORE was used to trim the low-quality reads. HISAT2 was applied to align the reads with the human reference genome HG38. Deseq2 was performed to discover differential gene expression between healthy and patient's dataset. Gene ontology (GO) and Kyoto Encyclopedia of Gene and Genome (KEGG) enriched pathways were also performed for annotation and visualization with DEGs.

Results: A total of 11 DEGs (with downregulated) were identified, including ATP11A, UTRN, Siglec1, XAF1, IFI44L, MX1, HERC5, SREK1, KDM7A, CELF2.AS1 and TAIL1, which were abnormally expressed in the GEO datasets. GO analysis in cellular component terms indicated that DEGs were mainly enriched in interferon signaling. Moreover, in biological processes where type I interferon signaling and cellular response to type 1 interferon were conferred. Also, in molecular function, DEGs were especially active in the filopodium membrane. KEGG pathway enrichment analyses demonstrated that DEGs were mainly enriched in histone demethylase activity and Phosphatidylserine flippase activity.

Conclusion: The multiple molecular mechanisms of these new vital genes in MS, ALS, and Sepsis need further investigation and may apply as prediction or diagnosis factors for treating these diseases.

Keywords: Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Sepsis, RNA-Sequencing, biomarker, differentially expressed genes, bioinformatical analysis

Immunology of Bacterial Infections

Seroprevalence trend of typhoid fever among patients referred to a medical center in southwest Iran, in 10-year periods

Sahar Molavi Nia[1], Abdolhussein Shakurnia[2], Mehri Ghafourian[2], Mehragin Mohmedi[3], Samaneh Salehipour bavarsad[4], Fatemeh Ahmadi[5]

1. Department of Medical Mycology, Faculty of Medical Sciences, Jundishapur University of Medical Science, Ahvaz, Iran.
2. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. Medical student, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4. Researcher of Gahade-daneshgahi Ahvaz, Hyperlipedia Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5. Department of Infectious and Tropical Diseases, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: Typhoid fever remains an important public health problem in the world, especially in the impoverished population of developing countries. Typhoid is one of the old endemic diseases of our country. Despite the efforts to control the disease, the disease has occurred in the country, but its prevalence is still considered in different climatic regions. The aim of this study was to explore the seroprevalence of typhoid fever among patients attending a Medical center, in Ahvaz over a 10-year period.

Methods: This was a retrospective, descriptive study carried out in laboratory-confirmed cases of typhoid fever during 2011–2020 from a medical center, in Ahvaz, Iran. Data records of patients who were clinically suspected of having typhoid fever diseases were analyzed. Initially, cases with typhoid fever were investigated in the laboratory by means of Widal agglutination tests. The widal serological test was considered positive 1/160 and above. Data were analyzed using SPSS-22 software and the chi-square test.

Results: Among the 8642 suspected patients within the 10-years, 88 (1.02%) were seropositive. Despite the trend of changes in positive cases from 0.4% in 2011 to 1.4% in 2015 and its zigzag diagram in different years, no statistically significant difference was observed between the positive cases by year (p=0.08). The lowest and highest seropositive frequencies were in summer and winter seasons (0.8% ver.1.2%); and in October and February months (0.4ver.1.6%), respectively (p=0.43). The frequency of seropositive cases of typhoid fever in women (1.07%) is higher than in men (0.97%), but this difference was not statistically significant (p=0.37). The highest frequency of seropositive cases was observed in the age group under 15 years (1.3%) and the lowest in the age group over 65 years (0.6%) (p=0.56).

Conclusion: The findings of this study can be of great value to health care policymakers in understanding the epidemiology of typhoid fever in this region

Keywords: Infectious diseases, Typhoid fever, Sero-prevalence, Seroepidemiology

A comparison between adjuvant and delivering functions of aluminum hydroxide and polyethylenimine nanoparticles, using a chimeric protein of Brucella Omp19-Omp31

Morteza abkar[1], Ali torkian[2]

1. Shahid Ashrafi Isfahani University, Isfahan, Iran.

Background: Brucellosis causes significant economic losses due to abortion, infertility, and decreased milk production. Vaccination against brucellosis is the most effective approach for preventing and controlling disease in endemic regions such as developing countries. Subunit vaccine, like recombinant proteins, which is appear to be safer than live attenuated strains, has been developed. Over recent years numerous protein antigens i.e., Omp31, Omp19 are shown to induce a protective immune response against brucellosis in mice model. In this study, chimeric protein containing Omp31-Omp19 (OO) along with aluminum hydroxide (AH/OO) and polyethylenimine (PEI/OO) nanoparticles (NPs) were compared to the immunogenicity in mice.

Methods: OO was expressed in E. coli and purification. NPs were synthesized and OO was loaded on to PEI and AlOH NPs The animals were immunized by subcutaneous injection with AlOH/OO or PEI/OO NPs. To analyze the antibody production, we took serum samples from the vaccinated animals. The total IgG, IgG1, IgG2a, IgM, and IgA isotype levels were examined against OO by an indirect ELISA method. The cytokines were assayed. For lymphocyte proliferation determination, the MTT assay was employed. Immunized groups of mice were challenged with the strain of B. melitensis 16M.

Results: In the present study, we evaluated the AlOH and PEI NPs ability as antigen delivery systems and adjuvants via subcutaneous administration route. The results of the antibody assay showed that the IgG titer after immunization with AlOH/OO NPs was higher than that of PEI/OO NPs. Our results indicate that immunization with PEI/OO and AH/OO NPs can significantly stimulate humoral immune response and induce secretion of cytokines. Hence, vaccination with PEI/Omp31 and AH/Omp31 NPs induces Th1-Th2 immune response. The results of the antibody assay and the protective results demonstrated that the recombinant AlOH/OO NPs had much greater protective effects against the B. melitensis challenge.

Conclusion: The obtained results demonstrated that AH and PEI NPs act as OO delivery systems and adjuvants for vaccination and protection against B. melitensis infection in mice. On the other side, AlOH/OO NPs had much greater protective effects.

Keywords: Brucellosis, chimeric protein, nanoparticles, aluminum hydroxide, polyethylenimine

Association between polymorphisms of cytokine genes and brucellosis: A comprehensive systematic review and meta-analysis

Parisa Zafari[1], Alireza Rafiei[1]

1. Mazandaran University of Medical Sciences, Sari, Iran.

Background: Owing to involvement of host genetic factors in susceptibility to brucellosis infection and its outcome, this study aimed to carry out a comprehensive systematic review and meta-analysis to derive a precise evaluation of the association between the risk of brucellosis and its focal complication and all cytokines examined in case-control studies, including Interferon-gamma (IFN-γ), Tumor Necrosis Factor (TNF)-α, TNF-β, Transforming Growth Factor(TGF)-β, IL-2, IL-4, IL-6, IL-10, IL-12B, IL-15, and IL-18 polymorphisms.

Methods: A systematic literature search in PubMed, Web of Science, Google Scholar, and Scopus was performed to identify the relevant studies, and related information was extracted. The effect size (ES) and corresponding 95% confidence intervals (CIs) were calculated to estimate the association.

Results: From 158 initial results, twenty-five eligible studies were included in the meta-analysis. Overall, the pooled results showed that the dominant models of IFN-γUTR5644, TGF-βrs1800470 and rs1800471, TNF-α rs1800629, and IL-10 rs1800872 were significantly less frequent in brucellosis patients than the controls. Also, the pooled analysis of the mutant allele vs. wild allele of TGF-βrs1800471 and IL-10 rs1800872 showed a negative association with brucellosis risk. On the other hand, our pooled analysis demonstrated that the mutant allele of IL-4 rs2243250 and IL-18 rs1946519 were associated with increased susceptibility to brucellosis. In addition, the IFN-γ UTR5644 and TGF-βrs1800470 were more frequent in the patients without focal forms.

Conclusion: IL-4 rs2243250 and IL-18 rs1946519 have a positive correlation with brucellosis whereas the IFN-γ UTR5644, TGF-β rs1800470 and rs1800471, TNF-α rs1800629, and IL-10 rs1800872 showed a negative association with this disease. The association between the other single nucleotide polymorphisms (SNP) and brucellosis risk was not confirmed in the current meta-analysis.

Keywords: Human brucellosis, Focal disease, Cytokine, Polymorphism, Allele, Genotype

Comparison of stool antigen test and IgG level in patients with Helicobacter pylori infection before and after treatment

Fatemeh Zare[1], Seyedeh Azra Shamsdin[1], Hossein Faramarzi[2]

1. Medical student at Shiraz University of Medical science, Shiraz, Iran.
2. Shiraz University of Medical science, Shiraz, Iran.

Background: H. pylori bacteria is known as the cause of the most common chronic infectious disease in the world. There are different methods to diagnose H. pylori, each of which has its own advantages and disadvantages. In the meantime, there is a difference of opinion about serological diagnostic methods, and their specificity and sensitivity are discussed, and the upcoming research has investigated this issue.

Methods: The study was started on 36 patients who had been referred to the Shahid Motahari Clinic in Shiraz with symptoms of dyspepsia and had a positive test for infection with H. pylori, and all patients were tested for stool antigen and anti H.pylori IgG titer at the beginning and end of the treatment. The treatment was done and finally, the data analysis was done with emphasis on comparison of the antibody titer between the patients who had successful treatment and the patients who remained infected.

Results: The average stool antigen before treatment was 4.2±0.46 and after treatment was 2.9±0.57, and a separate examination of its reduction rate in the two treated and untreated groups had a significant difference. Also, a comparison was made between the two groups in terms of the decrease in the anti H.pylori IgG. The treated group had a decrease of 9.44±1.78, while the antibody titer in the untreated group showed a decrease of 2.45±1.80. It was statistically significant.

Conclusion: The results obtained from the present study indicate a significant decrease in the Anti H.pylori IgG after treatment. Also, the average H.pylori stool Ag, which is an indicator of the bacterial load in the digestive system, was significantly reduced after treatment. It is worth mentioning that the antibody titer in the treated group that had a negative stool antigen test after treatment had a significant decrease compared to the untreated group.

Keywords: H. pylori, Stool antige, IgG antibody, infection

Creating and evaluation an animal model of sepsis (Acute inflammation) by the CLP method

Arezou khosrojerdi[1], Sara soudi[1]

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background: Sepsis is a systemic inflammatory disease in response to pathogens that leads to vital organ failures and the failure of vital organs. Appropriate animal models should be developed to measure the effectiveness of therapeutic methods. Cecal Ligation and Puncture (CLP) is the most widely used method of creating the sepsis model. Some variables interfered with the creation of the CLP model which terminated to result in an unrepeatable dynamic of the inflammatory responses. The current research, suggests presenting the simultaneous study of inflammatory responses in serum and liver as a criterion for determining the inflammatory status of the CLP model.

Methods: CLP model was induced in 15 female C57bl/6 mice. IL-6, TNF-α, IL-10, and TGF-β1 cytokines levels were measured at 24, 48, and 72 hours after CLP induction in both serum and liver tissue by ELISA method. Serum levels of liver enzymes were analyzed by the clinical chemistry analyzer. All studies were performed in healthy mice as well. The results were reported as Mean±SD.

Results: The levels of IL-10 and TGF- β1 in the liver were significantly ($P \leq 0.05$) higher than in serum. The production of IL-10 and TGF- β1 in the serum and liver reaches its maximum at peaked 24 and 72 hours after CLP induction. The level of TNF-αin the liver was significantly ($P \leq 0.05$) higher than in serum with a maximum production 24 hours after CLP induction.

Conclusion: Serum is not a good representative of the inflammatory condition in sepsis. Therefore, it is suggested that local inflammatory responses be considered in evaluating the model, and the determination of drug efficacy.

Keywords: Cecal ligation and puncture, Sepsis, Inflammation, Cytokine, Liver

Evaluation of serum ghrelin levels in patients with Helicobacter pylori infection

Seyedeh Azra Shamsdin[1], Seyedeh Neda shamsdin[2], Mahdi Bagheri[1], Fatemeh Sadeghi[1]

1. Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Basic Sciences in infectious diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Helicobacter pylori (H. pylori) is the main risk factor for stomach cancer and causes gastrointestinal diseases such as gastritis and peptic ulcer. Ghrelin hormone plays a role in regulating feeding behavior, energy metabolism and gastric acid secretion. So far, several possible reports have been published about the relationship between ghrelin and gastric cancer, this study investigated the relationship between H. pylori infection as the main risk factor for stomach cancer and changes in serum ghrelin levels.

Methods: This research is a cross-sectional study. Blood samples were collected from people with digestive problems who were referred to a gastroenterologist and liver specialist. The patients were selected with gastrointestinal symptoms and indigestion. 54 patients whose Helicobacter tests are positive and 36 patients whose tests are negative were included in the study. Serum ghrelin level was measured by the ELISA method.

Results: Ninety patients with gastrointestinal complaints have entered the study. Among the participants, 54 (60%) were women and 36 (40%) were men. The average age of the population was 37±11.5. Fifty-five people (60%) were positive for H. pylori infection and 36 (40%) were negative for H. pylori infection. The serum level of ghrelin was significantly higher in the group with a negative H. pylori test (2280.27±864.65 vs 1085.18±227.19, p=0.001). Among the clinic pathological indicators, patients with H. pylori infection and without infection had significant differences only in terms of hemoglobin (p=0.002) and hematocrit (p=0.008), and these two variables were significantly higher in the patients with H. pylori infection.

Conclusion: The findings of the present study indicated that the serum level of ghrelin in patients with H. pylori infection was statistically significantly lower than in the population without infection. Therefore, it seems necessary to conduct more studies in order to measure the clinical efficacy of serum ghrelin levels in the diagnosis of H. pylori infection.

Keywords: Serum, Ghrelin, Infection, H. pylori

Genetically Diverse Extensively and Pan Drug Resistant Pseudomonas aeruginosa main cause of nosocomial infection among hospitalized patients

Mina Boustanshenas[1], Bita Bakhshi[2], Ali Majidpour[1], Tahereh Mousavi Shabestari[1]

1. Iran University of Medical Sciences, Tehran, Iran.
2. Tarbiat Modares University, Tehran, Iran.

Background: Clinical strains of P. aeruginosa possess a wide diversity in antibiotic resistance and their genetic characteristics. The purpose of this study was to determine the antibiotic susceptibility pattern and genotyping of P. aeruginosa isolated from patients with nosocomial infections.

Methods: A total of 149 clinical isolates were collected from different wards of a referral hospital in Tehran, Iran. All of the samples were tested for isolation of P. aeruginosa and confirmed by PCR assay. The Multi-, Extensively- and Pan-Drug resistant strains were detected by CLSI guidelines. All isolates were subjected to ERIC-PCR genotyping using specific primers. The antibiotic patterns and ERIC-types were analyzed statistically using specific software.

Results: Seventy-six isolates (51%) were confirmed as P. aeruginosa among them 86.8% were determined as MDR, 81.5% as XDR and 5.3% as PDR. The highest resistance frequency was belonged to aminoglycosides, carbapenems, cephalosporins and fluoroquinolones, while polymyxin B was an effective antibiotic for treatment of our isolates. In our study, 8 different E-types were detected which belonged to 2 main clusters with a similarity rate of over 70%. The cluster B, composed of E-type G and H, was a dominant cluster and interestingly all of these cluster members were isolated from internal ICU, so we can claim at least 2 different colons have been colonized in internal ICU. Moreover, 4 PDR strains were detected in this study which 3 out of them possessed E-type G and the remaining was belonged to E-type H.

Conclusion: The heterogeneous population was observed among P. aeruginosa isolated in this study. Some of the unique E-types were dominant in ICUs with high diversity in their antibiotic resistance pattern which can be assumed as causative agents for nosocomial infection. The main threat that we should address here is regarding the PDR strains, since were isolated from the same ward and belonged to the same genetic cluster they could be considered as nosocomial pathogens and should be deliberated as a critical threat in emerging an outbreak in the hospital.

Keywords: Pseudomonas aeruginosa, ERIC-PCR, XDR, PDR

Investigating the level of anti- Acinetobacter baumannii antibodies and hospital infections in hemodialysis patients

Kasra Jahankhani[1], Saghar Chehrazi[2], Kobra Mahdinejadiani[3], Nariman Mosaffa[1]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Nephrology and Internal Medicine, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Department of Clinical Immunology, University of Calgary, Calgary, Canada

Background: Nosocomial infections are dangerous and patients who are hospitalized are exposed to infection. Acinetobacter baumannii is the most important hospital infection and can contaminate hospital equipment's and devices. Hemodialysis patients who are hospitalized for a long time and frequently undergo invasive interventions to perform hemodialysis are exposed to infection. In this research, we tried to determine the prevalence of the presence of anti-Acinetobacter baumannii antibodies, which can be an indication of the patient's infection and use it as a diagnostic tool to check the level of immunity in these patients and used to determine its relationship with clinical prognosis.

Methods: 20 hemodialysis patients were examined and compared with the control group. These patients had underlying diseases such as hypertension (11 patients), diabetes (2 patients), both diabetes and high blood pressure (3 patients), and heart problems (2 patients). Their vascular access was different such as SC/AVF, AVF, SHALDON, SC and catheter. Antibodies of Acinetobacter baumannii were examined through specific ELISA kit.

Results: Compared to the non-expose control group, the cut off was 0.406, and we considered people above this value to be positive. 14/20 patients were higher and 6/20 were lower. Among positives, 11/14 had high blood pressure, 2/14 had diabetes, 3/14 had both diabetes and high blood pressure and 1/14 had heart problems. 2/2 and 3/4 patients whose vascular access was SC and SHALDON had high blood pressure and antibodies.

Conclusion: Most of the patients in whom the antibody was higher than the cut-off, had high blood pressure, which can indicate contamination in these patients. For most of these patients who had hypertension, their vascular access was SC or SHALDON, which can show that these access ways can increase the risk of infection. The presence of antibodies can indicate protection, and its lack can indicate the inability to produce antibodies due to immune deficiency.

Keywords: Acinetobacter baumannii, hemodialysis, Nosocomial infections, blood pressure

Novel mouse monoclonal antibodies against Bordetella pertussis pertactin (PRN) antigen with versatile applications

Danyal Imani[1], Tannaz Bahadori[1], Sedighe Ghourchian[2], Forough Golsaz-Shirazi[1], Masoumeh Douraghi[2], Mahmood Jeddi-Tehrani[3], Mohammad Mehdi Amiri[1], Fazel Shokri[1]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Monoclonal Antibody Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran

Background: Pertussis, or whooping cough, is a highly contagious respiratory disease caused by Bordetella pertussis (B. pertussis). Pertactin (PRN) antigen is one of the immunogenic components in many commercialized acellular pertussis vaccines (aPVs). Conventional PRN antigen purification is challenging and commonly needs multiple laborious processes with low recovery. Using specific monoclonal antibodies (mAbs) to purify PRN antigen is expected to yield high purity and recovery of the target molecule.

Methods: The recombinant PRN antigen was used to produce mouse monoclonal antibodies using hybridoma technology. Structural and functional characteristics of mAbs were assessed by ELISA, immunoblotting, and flow cytometry. The affinity column chromatography using these monoclonal antibodies was employed to purify PRN antigen and purity and recovery were then analyzed by ELISA and SDS-PAGE. Moreover, ELISA and flow cytometry techniques were designed by these mAbs to investigate the recognition of PRN antigen and different strains of B. pertussis.

Results: Five mAbs were produced based on selection by native form of PRN. Our results demonstrated that purification of PRN by affinity chromatography column resulted in a pure antigen with a recovery rate of about 80-85 percent. In addition, ELISA and flow cytometry results indicated that the produced mAbs could recognize native antigens in the bacterial cell wall of different B. pertussis strains (BP 134, Tohama I, and BP509).

Conclusion: We successfully produced PRN-specific mAbs and designed an affinity chromatography method to purify PRN antigen with high purity and recovery than conventional methods. These mAbs could be employed as valuable tools for diagnostic purposes.

Keywords: Bordetella pertussis, Pertactin, Monoclonal antibody, affinity chromatography, diagnosis

Serological evaluation of horse specimens suspected of glanders using Western blot

Nafiseh Shakibamehr[1], Nader Mosavari[1], Shojaat Dashtipoor[1], Samerand Reshadi[1], Mahdi Babaei[1]

1. Reference Laboratory of Bovine Tuberculosis, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran

Background: Burkholderia mallei, is the etiologic agent of the glanders disease. Clinical and bacteriological diagnosis of glanders is difficult in the early stages of the disease. Some methods such as Complement fixation test (CFT) due to false positive results and troublesome for veterinary authorities and cause financial losses to animal owners. The other one is Malleination test, which requires appropriate equipment and efficient laboratory personnel. Therefore, in order to quickly and accurately diagnose the disease, especially in areas that cannot be kept animals, new methods should be used to identify the disease.

Methods: The Western blot is a serological diagnostic test and has been recommended by the World Organization for Animal Health (OIE). In this study, a Western blot assay making use of a purified lipopolysaccharide (LPS) containing antigen of Burkholderia mallei was designated. In this study, a total of 75 sera were collected from different horse populations from several geographical areas of Iran. Specificity and sensitivity of the Complement fixation test, ELISA and a Western blot were compared for serodiagnosis of glanders. ELISA test was based on B. mallei antigens and Western blot made use of a purified LPS-containing B. mallei-antigen. The Western blot and ELISA, were more specific than the Complement fixation test. ELISA based on B. mallei antigens had more sensitivity compared to Complement fixation test and Western blot.

Results: Finally, sensitivity and specificity were obtained for Complement fixation test (92.31% and 98.38%), Western blot (92.31%, 100%) and (100% and 100%) respectively. Complement fixation test for glanders is still the prescribed serological method for commercial purposes, which is used to confirm the health of animals. However, in order to maintain the biosafety of valuable and expensive horse colonies, it is important to implement a more accurate and intelligent disease control and eradication program.

Conclusion: This enhances the laboratory diagnostic capabilities to better understand the cause of the disease and to use and optimize the diagnostic methods of glanders in the country. Therefore, efforts to further improve and optimize ELISA and Western blotting should be continuing.

Keywords: Burkholderia mallei, glanders, western blot

Seroprevalence of laboratory-confirmed Bordetella pertussis antibodies after Immunization in Iran: a systematic review and meta-analysis of healthy-population study

Ali Rahimi[1]

1. Iran University of Medical Sciences, Tehran, Iran.

Background: Pertussis (whooping cough) which causes by Bordetella pertusis (BP), is one of contagious upper respiratory infection and also vaccine preventable disease with high morbidity and mortality. Based on the immune response of the host, the severity of BP infection is variable, therefore, analyzing the BP seroprevalence rate in the general population may improve the implementation of new strategic health management such as developed vaccination schedule. Therefore, we aimed to provide a systematic review of the seroprevalence rate of BP infection among Iranian population through estimating the prevalence of BP according to age, gender, infancy and pre- and post-booster vaccination.

Methods: A thorough systematic literature was performed in databases PubMed, Embase, Web of Science, Scopus, and Google Scholar and also in national Persian databases up to October 2020 to identify eligible studies evaluating seroprevalence of specific antibodies for BP in healthy individuals in Iran. Finally, out of 172 total studies, 28 relevant studies including healthy individuals in the age-range between 0-67 years were participated in this meta-analysis. Heterogeneity test of the selected studies was calculated using I2 statistic. The random effect model was used based on heterogeneity test results and pooled data expressed as the effect size (ES) with 95% confidence intervals (CIs).

Results: The overall IgG seroprevalence rate of BP infection in general population of Iran was 50% (95% CI; 43%-57%) and the overall IgA seroprevalence rate was 20% (95% CI; -2%-43%). Also, the Total IgG seroprevalence rate among infants was 38% and there is no significant seroprevalence difference between females and males (39% vs 41% respectively). Furthermore, the relative rate of seropositivity is high in children (54%, 95% CI; 42%-66%) and post-booster vaccinated individuals (77%, 95% CI; 59%-94%).

Conclusion: Our findings may provide a better image of background and assessment of BP infection in Iran and promote a schedule for cost-benefit immunization. Further consideration to disease seroprevalence is recommended by the subsequent data in order to gather effective information for clinical intervention targeted against BP.

Keywords: Bordetella Pertussis (BP), Seroprevalence, IgG, Vaccination, Immunity, Iran

The Most Common Serological Tests for Helicobacter pylori Infection

Nazanin Moghaddam[1], Sina Moghaddam[2], Maryam Meskini[2]

1. Laboratory of Medicinal Chemistry, Department of Clinical Research, Pasteur Institute of Iran, Tehran, Iran
2. Department of Mycobacteriology and Pulmonary Research, Pasteur Institute of Iran, Tehran, Iran

Background: As a gram-negative microaerophile, Helicobacter pylori is considered pathogenic. One of the most common infections in humans is Helicobacter pylori, which is responsible for chronic diseases such as chronic active gastritis, ulcers and mucosal tissue-related lymphomas (MALT). The risk of ulcers and gastric cancer can be partially prevented by eradication or vaccination. On the other hand, several scientists have suggested that the co-evolution of Helicobacter pylori with the human population may indicate the beneficial effects of this bacterium on the host, which protects against childhood diarrhea.

Methods: The PubMed, Google Scholar and Scopus databases were searched to find studies and fifteen articles were studied.

Results: IgG and IgA antibodies are used to detect Helicobacter pylori infections. Helicobacter pylori can be diagnosed with IgG, and enzyme immunoassay (EIA) is the most common and accurate method. Assays such as immunoblots, Luminex-based bead assays, and line assays that specialize in bacterial pathogens and the host's immune response have been developed recently. Infection is diagnosed based on pathogenic proteins such as OMP, CagA, ureA, VacA, and GroEl.

Conclusion: In studies of various Helicobacter pylori antigens, researchers have found a connection between a positive immune response and clinical outcomes such as gastric cancer, intestinal metaplasia, and atrophic gastritis.

Keywords: Helicobacter pylori, Gastric ulcer, Serological

Tuberculosis result of host mycobacterium interaction; evaluating of host defense and bacteria virulence factors expression

Fatemeh Sadat Mohammadi[1], Sara Hajimiri[2], Nazila Ariaee[3], Seyed Abdolrahim Rezaee[3]

1. Immunology Research Center, Inflammation and Inflammatory Diseases Division, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Antimicrobial Resistance Research Center, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran.Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Immunology Research Center, Inflammation and Inflammatory Diseases Division, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Mycobacterium tuberculosis (M. tb) could render type IV delayed hypersensitivity reaction. The production of matrix metalloproteinase (MMPs) and leukocyte chemotaxis toward the site of infection are important aspects of the immunological pathogenesis of tuberculosis (TB).

Methods: Bronchoalveolar lavage (BAL) specimens were utilized to separate mononuclear cells, and target genomic DNA was employed for qPCR TB diagnosis and cDNA for specific RT-qPCR gene expression. The subjects were then classified into TB+ and TB- groups, and the expression levels of CFP-10, ESAT-6, CCR1, CCR12 and MMP3, 9 were evaluated.

Results: The mean level of CCR1 expression in TB+ and TB- patients' BAL was 1.71 ± 0.78 and 0.5 ± 0.22, respectively, which was statistically different ($p = 0.01$). The CCR2 level, in TB+ (2.07 ± 1.4), was higher than in TB- patients (1.42 ± 0.89, p = 0.01). The MMP9 expression in TB+ was 2.56 ± 0.68, also higher than in TB- patients (1.13 ± 0.35), while MMP3 was lower in TB+ (0.22 ± 0.09) than in TB- (0.64 ± 0.230, $p = 0.05$). The CCR2/CCR1 and MMP3/MMP9 balance in TB+ were reduced, compared to the TB-. The CFP-10 and ESAT-6 were highly expressed in TB+ patients. The CFP-10 expression had a strong negative correlation with albumin (r = - 0.93, $p = 0.001$), and a negative correlation with neutrophil (r = - 0.444, $p = 0.1$ with 90% CI). The MMP-9 expression showed a positive correlation with WBC count (r = 0.61, p = 0.02), in TB+, and had a negative correlation with BMI (r = 0.59, $p = 0.02$) in TB-. The M. tb CFP-10 may be responsible for reducing MMP3 and CCR2 expression to aid in M. tb spread.

Conclusion: Moreover, the proportions of MMP3/MMP9 and CCR2/CCR1 can be used as predictive indicators of the severity of TB.

Keywords: CCR; CFP-10; ESAT-6; Gene expression; MMP; Mycobacterium tuberculosis (M.tb); Tuberculosis.

Immunology of Fungal Infections

Comparing the in vitro activity of seven antifungal drug with Chlorhexidine against most important causative agents of fungal keratitis

Afsaneh Mohammadi[1], Sadegh Khodavaisy[1], Alireza Izadi[3], Claudy Oliveira dos Santos[2], Marlou C. Tehupeiory-Kooreman[2], Mohammad Soleimani[3], Zohre Abedinifar[3], Paul Abedinifar[2]

1. Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Centre for Expertise in Mycology, Department of Medical Microbiology, Radboud University Medical Center, 6525 GA Nijmegen, the Netherlands
3. Department of Ocular Trauma and Emergency, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran.

Background: The treatment of supportive fungal keratitis (FK) caused by fungi is challenging especially in developing countries, Early and adequate treatment of FK is essential to prevent disease progression and blindness. The aim of the study was to investigate the in vitro activity of chlorhexidine (CHX) and seven antifungal agents against a collection of fungi recovered from patients with FK.

Methods: Antifungal susceptibility testing was performed according to the EUCAST broth microdilution reference method. The antifungal agents tested included fluconazole (range 64–0.06 µg/ml), voriconazole (range 16–0.03 µg/ml), posaconazole (range 16–0.03 µg/ml), miconazole (range 16–0.03 µg/ml), natamycin (range 16–0.03 µg/ml), amphotericin B, and caspofungin (range 8–0.008 µg/ml). For chlorhexidine, a concentration range of 1 to 1,024 µg/ml was used.

Results: CHX showed the most favorable in vitro inhibition of filamentus and yeast fungi. CHX showed activity against Fusarium species, Aspergillus species, Candida species at concentrations of 0.004-0.125, 0.016-0.063, and 0.004-0.031 µg/ml respectively. Posaconazole was the most active antifungal agent followed by voriconazole, amphotericin B and caspofungin.

Conclusion: CHX should be further evaluated as a first line treatment for FK in settings where microbiological facilities and a variety of antifungal drugs are not readily available. It may have potential as an affordable topical agent for the condition.

Keywords: Fungal keratitis, Chlorhexidine, Antifungal, Fusarium

COVID-19-associated aspergillosis and mucormycosis: Case series and comprehensive review

Elahe Sasani[1]

1. Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Background: Mucormycosis and aspergillosis are angioinvasive infections occurring mainly in immunocompromised patients. However, mixed infection with mucormycosis and aspergillosis in post-COVID-19 patients is rare. Here, we will report four cases and review comprehensively published literature on COVID-19 infection associated with aspergillosis and mucormycosis.

Methods: In addition to four of our cases, we searched published articles using PubMed/MEDLINE, Scopus, and Web of Science databases from the beginning of 2020 to November 2022.

Results: The mean age of 51 analyzed cases (4 of our and 47 other cases) was 57.9 years. The most common underlying disease (58.8%) was diabetes mellitus. However, nine COVID-19 patients had no underlying condition. The primary diagnosis of aspergillosis (17.6%) before mucormycosis was led to the voriconazole prescription and followed by a change to amphotericin B. The overall mortality rate was 37.2%.

Conclusion: This study highlights the presence of mixed fungal infections in COVID-19 patients who previously had common underlying diseases or even a healthy immune system. Therefore, evaluation of the cumulative-corticosteroid doses used, as a risk factor, during Covid-19 treatment is important.

Keywords: Aspergillosis; Mucormycosis; COVID-19

Immunological aspects of fungal infections in burn patients

Mohammad Ali Bahar[1], Hadi Amrollahi[2]

1. Iran University of Medical Sciences, Tehran, Iran.
2. Semnan University of Medical Sciences, Semnan, Iran.

Background: Burn injury is a serious condition that predisposes patients to fungal infections. Fungal infections are often difficult to diagnose and treat due to their complex immunological mechanisms. The aim of this study is to review the immunological aspects of fungal infections in burn injury patients.

Methods: Fungal infections are recognized by pattern recognition receptors (PRRs) on the surface of innate immune cells, such as dendritic cells and macrophages. These cells respond by secreting cytokines and chemokines, recruiting other immune cells to the site of infection, and initiating phagocytosis of the invading fungus. The adaptive immune response is critical for the clearance of fungal infections, involving the activation of T cells and B cells, which produce antibodies and coordinate the response of other immune cells. However, some fungi have evolved strategies to evade immune detection or subvert immune responses, leading to persistent infections.

Results: Fungal infections are a common complication in burn injury patients and are associated with high morbidity and mortality. The immune response to fungal infections in burn injury patients is complex, involving multiple components of the immune system. These include innate immune cells such as neutrophils, macrophages, and dendritic cells, as well as adaptive immune cells such as T cells and B cells. The host response to fungal infections in burn injury patients is often impaired, leading to a poor prognosis.

Conclusion: Fungal infections in burn injury patients are a significant clinical problem that requires early diagnosis and prompt treatment. Understanding the immunological mechanisms involved in fungal infections in burn injury patients can aid in the development of effective treatments and improve patient outcomes. Clinicians should consider fungal infections in the differential diagnosis of burn injury patients, especially those who do not respond to standard therapies.

Keywords: Immunological aspects, Fungal Infections, Burn patients

Impact of silver nanoparticles on Aspergillus fumigatus and aspf1 gene expression

Bahareh Bashardoust[1]

1. Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: Removing Aspergillus fumigatus conidia for allergy and infection prevention and control from the environment, especially the intensive care units of hospitals, is a challenge. Furthermore, the emergence of multidrug resistance-Aspergillus is an important problem in healthcare. To tackle these challenges, efficacy evaluation of silver nanoparticles (Ag-NPs) on Aspergillus fumigatus and expression of the aspf1 gene that involves in allergic and invasive aspergillosis can be beneficial.

Methods: Antifungal susceptibility test on Aspergillus fumigatus ATCC204305 and the clinical isolate was performed using the micro-titer broth dilution method. The expression of the aspf1 gene before and after exposure to Ag-NPs was measured using real-time PCR.

Results: The minimum inhibitory concentration (MIC90) and the minimum fungicidal concentration (MFC) of Ag-NPs against Aspergillus fumigatus isolates were 62.5, 125, and 125, 250 µg/mL, respectively. The expression ratio of the aspf1 gene in comparison to β-tubulin as a reference gene in Aspergillus fumigatus isolates after exposure to Ag-NPs were 0.007 and 0.5, respectively (P-value < 0.05).

Conclusion: It can be concluded that Ag-NPs have high potency of antifungal activity against Aspergillus fumigatus isolates and reduce aspf1 gene expression.

Keywords: Aspergillus fumigatus, silver nanoparticles, Antifungal susceptibility, Quantitative Real-Time PCR

Immunology of Rheumatic Diseases

Analysis of the Effect of MMP-9 -1562C/T Gene Polymorphism on MMP-9 Serum Level in Rheumatoid Arthritis Patients

Roghaye Keshavarz Sadegh[1], Sanaz Keshavarz Shahbaz[2], Maryam khalaji[3], Mehdi Sahmani[4], Mahin Lashgari5, Amir Javadi[6], Farshad Foroughi[7]

1. Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran
2. Cellular and Molecular Research Center, Research Institute for Prevention of Non-communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran
3. Department of Biochemistry & Genetics, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
4. Department of Clinical Biochemistry and Genetics, Faculty of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
5. Metabolic Disease Research Center, Research Institute for Prevention of non-Communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran
6. Department of Social Sciences, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
7. Department of Immunology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran; 2. Cellular and Molecular Research Center, Research Institute for Prevention of Non-communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran

Background: Rheumatoid arthritis (RA) is a systemic inflammatory disease that principally affects synovial joints, causing progressive cartilage and bone damage. Matrix metalloproteinases (MMPs) are the key enzymes in the pathogenesis of cartilage and joint destruction, considered a new biomarker of the early erosive form of RA. In this way, we studied the effect of the MMP-9-1562C/T gene polymorphism on the serum levels of this enzyme in RA.

Methods: RA patients were confirmed utilizing RF (rheumatoid factor), Anti CCP (antibody against cyclic citrullinated peptide), and CRP (C-reactive protein). The MMP-9 serum levels were measured utilizing the enzyme-linked Immunosorbent assay (ELISA). Then the MMP-9-1562C/T gene polymorphism was analyzed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Finally, we studied the association between MMP-9-1562C/T gene polymorphism and the risk of RA.

Results: The current study showed that the levels of inflammatory factors in RA patients are significantly higher than in the control group. Also, the increase of MMP-9 serum levels in patients due to the MMP-9-1562C/T gene polymorphism was confirmed by increasing the heterozygous genotype (CT) frequency. Correlation analysis indicated a positive connection between the CT genotype and susceptibility to RA. Logistic regression analysis also confirmed that the chance of developing RA is higher in people with CT/CC genotype than in other alleles.

Conclusions: The current case-control study showed that the MMP-9-1562C/T gene polymorphism is associated with the serum levels of MMP-9 and the susceptibility of RA.

Keywords: Rheumatoid arthritis (RA), Matrix metalloproteinase-9 (MMP-9), MMP-9-1562C/T gene polymorphism, MMP-9 serum levels

Anti-inflammatory and Anti-oxidant Effects of Punica Granatum and Berberis Vulgaris Juice on Adjuvant-induced Arthritis Model in Rats

Maryam Mohammadi[1], Gholamreza Anani Sarab[1], Mahdi Honarbakhsh[1], Melika Hasani[1]

1. Birjand University of Medical Sciences, Birjand, Iran.

Background: Rheumatoid arthritis (RA) is a complicated autoimmune problem affecting numerous joints in the body. Because of severe undesirable effects of chemical medications, searching for novel herbal remedies with suitable therapeutic effects and less side effects is continued. The aim of this study was to evaluate Anti-inflammatory and anti-oxidant properties of Punica granatum and Berberis vulgaris juice on Complete Freund's Adjuvant (CFA) model of rheumatoid arthritis in rats.

Methods: Thirty-six male Wistar rat (150-200g) were divided into 6 groups: healthy control (HC), CFA, positive control treated by Dexamethasone (DXM), positive control treated by Methotrexate (MTX), pomegranate juice (150mg/kg) and Berberis juice (150 mg/kg). Rheumatoid arthritis was induced by single sub-plantar injection of Freund's Complete Adjuvant (0.1mL, CFA) into the left hind paw on day 0.Every five days, the severity of the clinical signs of rheumatoid arthritis include erythema and swelling which are considered as an arthritis Index were assessed via standard scoring system. Moreover, the rats' hind paw edema was measured by digital vernier caliper. On day 28, rats were anesthetized and sacrificed then serum was collected and the level of oxidative stress parameters (TAC and MDA) were evaluated.

Results: The study indicated that administration of pomegranate juice can significantly reduce arthritis index on day 28th in comparison with CFA group ($p< 0.024$).On the other hand, Berberis juice has shown no significant decrease in arthritis score and hind paw edema. In terms of oxidative stress markers, Berberis juice significantly increased Total Anti-oxidant Capacity (TAC) of serum compared to CFA ($p<0.042$).

Conclusion: Regarding the results, pomegranate juice could decrease the severity of clinical signs in animal RA model. Besides, Berberis juice have potential anti-oxidant activity.

Keywords: Adjuvant-induced arthritis, Rheumatoid arthritis, Punica granatum, Berberis vulgaris,

Anti-inflammatory Effects of Lactobacillus Delbruki and Lactobacillus Rhamnosus Probiotics on Phenotype and Gene Expression of M1 and M2 Macrophages Produced from Monocytes of New Case Lupus Patients

Zahra Javanmardi[1], Mahmoud Mahmoudi[2], Houshang Rafat Panah Bayegi[2], Zahra Rezaie yazdi[3], Nafiseh sadat Tabasi[2], Abbas Shapouri Moghaddam[4], Parisa ahmadi[5], Sara Hosseini[1], Seyed-alireza Esmaeili[2]

1. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
2. Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Rheumatic Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
4. Stem Cell and Regenerative Medicine Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Systemic lupus erythematosus is a complex autoimmune disease which self-tolerance breakdown plays key role in its onset and development. Macrophages are involved in pathogenesis through defective phagocytosis of dead cells or M1/M2 imbalance. Also, the immunomodulatory effects of Lactobacillus delbrueckii and Lactobacillus rhamnosus have been proven. So, the aim of study was to investigate effects of Lactobacillus delbrueckii and L.rhamnosus on M1 and M2 macrophages differentiation in lupus patients.

Methods: First, blood monocytes were collected from lupus (3 people) and healthy (3 people) individuals and to produce macrophages, were cultured for 5 days. Then, macrophages were cultured in the presence of probiotics or LPS for 48 hours. Finally, the expression of CD14, CD80 and HLADR macrophage markers was analyzed by flowcytometry and expression of their cytokines (IL1-β, Il-12, TNF-α, IL-10 and TGF-β) was analyzed by Real-Time PCR method.

Results: The results showed that macrophages were composed of three populations M0, M1, and M2. In both control and patient groups, a decrease in CD14, CD80, and HLA-DR expression in probiotic groups was seen compared to LPS group. This diminished effect in M0 and M2 macrophages of lupus subjects were more than healthy subjects, but in the case of M1 macrophages, was more in healthy subjects. Also, increased levels of IL-10 and TGF-β and decreased levels of IL-12, IL1-β, and TNF-α was seen in probiotic groups of healthy and lupus people compared to the LPS group. The expression of inflammatory cytokines was higher in lupus patients, but expression of anti-inflammatory cytokines was higher in healthy subjects.

Conclusion: In general, Lactobacillus delbrueckii and Lactobacillus rhamnosus were able to have anti-inflammatory effects on macrophages of healthy and lupus patients. So, probiotics may be able to reduce the inflammation in lupus patients.

Keywords: Probiotics, Lactobacillus delbrueckii, Lactobacillus rhamnosus, Macrophage

Asymmetric and Symmetric Dimethylarginine Concentration as an Indicator of Cardiovascular Diseases in Rheumatoid Arthritis Patients: a Systematic Review and Meta-analysis of Case-control Studies

Parisa Zafari[1]

1. *Mazandaran University of Medical Sciences, Sari, Iran.*

Background: Rheumatoid arthritis (RA) is the most common type of inflammatory arthritis leading to joint damage and physical disability. Cardiovascular diseases (CVDs) are considered common comorbidity in patients with RA. However, the mechanism underlying its pathogenesis is not definitively explained. Endothelial dysfunction caused by impaired nitric oxide synthesis is an early indicator of cardiovascular disease. Asymmetric and symmetric dimethylarginine (ADMA and SDMA, respectively) the inhibitors of endothelial nitric oxide synthase (NOS) have emerged as novel CVD risk factor determiners. Concerning the unmet need to identify a salutary biomarker for CVD prediction, the purpose of this meta-analysis was to assess the serum/plasma ADMA and SDMA levels in RA patients compared with the healthy controls.

Methods: A thorough literature search was performed in PubMed, Scopus, Web of Science, and Google Scholar to identify all studies reporting ADMA and/or SDMA levels in RA patients compared with healthy controls. The quality of studies was evaluated using the Newcastle-Ottawa scale (NOS). Pooled standard mean difference (SMD) with 95% confidence interval (CI) was used as the effect size in this study. We also conducted stratified analysis based on assay methods and median age of the participants.

Results: Fourteen articles were included. The pooled serum/plasma levels of ADMA were higher in RA patients compared with those of healthy controls (SMD= 1.02, 95% CI = 0.49 to 1.55); However, no statistical differences between RA patients and healthy controls in serum/plasma SDMA levels was seen (SMD= 0.57, 95% CI = −0.21 to 1.36). Subgroup analyses suggested that participants aged > 50 years had higher levels of ADMA rather than controls and the measurement method was a source of heterogeneity for ADMA.

Conclusion: ADMA measurement but not SDMA, can be useful for assessment of endothelial dysfunction as a predictor of CVD risk in RA patients.

Keywords: Atherosclerosis, Cardiovascular diseases, endothelial dysfunction, Rheumatoid arthritis, Symmetric dimethylarginine

Dimethyl Fumarate Inhibits Fibroblast like Synoviocytes-mediated Inflammation and Joint Destruction in Rheumatoid Arthritis

Parisa Zafari[1], Mahdi Taghadosi[2], Fatemeh Faramarzi[1], Misagh Rajabinejad[1], Alireza Rafiei[1]

1. Mazandaran University of Medical Sciences, Sari, Iran.
2. Kermanshah University of Medical Sciences, Kermanshah, Iran.

Background: Rheumatoid arthritis (RA) as a chronic inflammatory disorder affects around 1% of the world population. Fibroblast-like synoviocyte (FLS), one of the main cells in RA pathogenesis is characterized by hyperproliferation and resistance to apoptosis resulting to synovial hyperplasia. Dimethyl fumarate (DMF) has been licensed for the treatment of multiple sclerosis (MS) and psoriasis; however, its role in RA is unknown. DMF has immunomodulatory properties and may be considered as therapeutic approach in RA treatment. In this study, we aimed to investigate the effect of DMF on controlling FLS-mediated synovial inflammation and joint destruction in RA.

Methods: FLSs were isolated from synovial tissues of 8 patients with RA and treated with DMF. Apoptosis rate was analyzed by Annexin V-FITC. Cell proliferation was measured by carboxyfluorescein succinimidyl ester (CFSE) dye. The matrix metalloproteinase 3 (MMP3) and NF-κB pathway protein (p65) mRNA expression were evaluated by RT-PCR. Also, the IL-6 production and lactate release were measured in FLS supernatant.

Results: DMF treatment decreased the cell proliferation and increased apoptosis in a dose dependent manner. DMF-treated FLS showed a reduction in IL-6 and lactate release. Moreover, it was revealed that DMF inhibited the expression of p65 and MMP3.

Conclusion: Our data demonstrate that DMF treatment suppresses the aggressive and inflammatory features of RA FLSs. Our Results suggest that DMF might be expected to be evaluated as a therapy for RA.

Keywords: Fibroblast like synoviocyte, Rheumatoid arthritis, Inflammation, Dimethyl fumarate

Dimethyl Fumarate Suppresses Inflammation and Ameliorates Collagen-Induced Arthritis in Lewis Rats

Parisa Zafari[1], Misagh Rajabinejad[1], Alireza Rafiei[1]

1. Mazandaran University of Medical Sciences, Sari, Iran.

Background: Dimethyl fumarate (DMF) is licensed for use in the treatment of autoimmune and inflammatory diseases; however, its role in the RA has not well been examined. In this study, a collagen-induced arthritis (CIA) rat model was employed to evaluate the effect of DMF on RA in vivo.

Methods: Bovine type II collagen emulsified with incomplete Freund's adjuvant (IFA) was used to stablish a model of arthritis in Lewis rats. The rats were randomly divided into five groups: normal control, CIA control, vehicle, arthritic rats treated with DMF, and MTX. Clinical evaluation was performed via measurement of hind paw swelling and arthritis scores. On day 28, rats were sacrificed and hind ankle joints were gathered for histopathological evaluation and after that for radiography. Also, serum level of TNF-α and anti-collagen type II was measured by ELISA.

Results: In vivo results showed that DMF significantly decreased CIA-induced arthritis score, hind paw swelling, cartilage destruction, and joint inflammation. The level of anti-collagen type II and TNF-α was lower in DMF treatment group compared with CIA control.

Conclusion: DMF ameliorated RA and this effect may be related to its immunoregulatory function. The use of DMF as a potential novel therapeutic agent in the management of RA could be investigated.

Keywords: Animal model, Collagen induced arthritis, Dimethyl fumarate, Inflammation, Rheumatoid arthritis

Effects of the Fibroblast Activation Protein Inhibitor, Talabostat, on the Expression of Mediators that are Involved in the Fibrosis and Inflammation in Systemic Sclerosis

Mehrnoosh Pashaei[1], Elham Farhadi[2], Hoda Kavosi[2], Mahdi Mahmoudi[2], Aliakbar Amirzargar[1]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Systemic sclerosis (SSc) is a connective tissue disease characterized by vasculopathy and immune dysregulation which leading to fibrosis. Fibroblasts play an important role in the progression of fibrosis through the expression of extracellular matrix (ECM) and matrix metalloproteinases (MMPs), which degrade ECM. Fibroblast activation protein (FAPα) is a member of the cell surface dipeptidyl peptidase (DPP) family of serine proteases which expressed on activated fibroblast involved in tissue remodeling in fibrogenic processes. However, the role of FAPα in tissue fibrosis is controversial, and data on pharmacological FAP inhibition are lacking. The present study aims to evaluate the effect of Talabostat, a small molecule inhibitor of FAP-α, on fibrosis and inflammation in SSc pathogenesis.

Methods: Dermis fibroblasts were isolated from skin biopsies of 10 diffuse cutaneous SSc and 10 healthy subjects. The isolated cells were confirmed by immunofluorescence staining and microscopically. Fibroblasts are treated with transforming growth factor β (TGF-β) that acts like inducer of FAPα expression and Talabostat. Then, the expression of ECM, MMPs, and inflammatory-related genes (such as FAPα, COL1A1, COL1A2, IL6, TGFB1, MMP1, MMP2, MMP9, and ACT2) and α-SMA protein were analyzed by Real-time PCR and immunocytochemistry.

Results: FAPα expression had no difference between in explanted SSc dermal fibroblasts and matched healthy subjects. However, FAPα, COL1A1, and IL6 mRNA expression significantly was up-regulated by TGF-β in explanted SSc dermal fibroblasts compared with healthy subjects. On the other hand, down-regulation of FAPα, COL1A1, COL1A2, and IL6 and also up-regulation of MMP9 expression were observed by Talabostat in dermal fibroblasts of SSc patients. These gene expression changes were not observed in healthy subjects.

Conclusion: This study indicate the role of FAP-α in fibrosis and the effect of Talabostat on it in SSc patients and prospects for the treatment of fibrosis in these patients in the future.

Keywords: Systemic sclerosis, FAP-α, Talabostat, Fibrosis

Efficacy and Safety of Rituximab Therapy in Patients with Systemic Sclerosis Disease (Ssc): Systematic Review and Meta-Analysis

Maliheh Mradzadeh[1], Zahra Arab-Bafrani[1]

1. Golestan University of Medical Sciences, Golestan, Iran.

Background: The clinical benefits of rituximab in systemic sclerosis (SSc) are still contentious. The present meta-analysis aimedto systematically assess rituximab's safety and efficacy profile in SSc patients.

Methods: A systematic online query was performed in PubMed, Scopus, Web of Science, and Embase. The studies on the application of rituximab for patients with SSc were reviewed comprehensively for over two years. In terms of efficacy profile, mRSS, MS, LVEF, sPAP, FVC, DLCO, TLC, FEV, DAS, severity activity, HAQ-DI and SF36 were assessed for organ involvement and quality of life. The level of biological and immunological markers was also evaluated in SSc patients treated with RTX. In total, 24 studies met the criteria. Although they did not have a high quality, they were free from heterogeneity and publication bias.

Results: The pooled results revealed a long-term improvement in mRSS and MS.HAQ-DI was improved to 0.78 after 12 months, and DAS was significantly reduced to 0.33, 0.23, and 0.24following 6, 12, and 24 months of treatment, respectively (p=0.001 for both parameters). The rest of the parameters remained stable over time in patients with SSc. The pooled analysis of these patients demonstrated that the induction of death, cancer, infection, and infusion were 9, 5, 18 and 10%, respectively.

Conclusion: Based on the pooled results of this meta-analysis, rituximab improves skin score and disease indices and stabilizes organ involvement in SSc patients. Rituximab seems to possess reasonable safety, similar to previous data from other autoimmune diseases.

Keywords: Efficacy, Organ involvement, Rituximab, Safety, Skin function, Systemic sclerosis

Evaluation of Salirasib Effect on the Expression of Genes Involved in Fibrosis in Skin Fibroblasts of Systemic Sclerosis Patients

Mohammad Vodjgani[1], Mina Sadeghi[1], Elham Farhadi[2], Mahdi Mahmoudi[2], Mohsen Rokni[1], Samaneh Enayati[2], Hoda Kavosi[2]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Systemic sclerosis (SSc) is an autoimmune disease characterized by immunological abnormalities, vascular damage, inflammation, and fibrosis. Tissue fibrosis plays an important role in SSc and can affect several organs such as the skin, joints, tendons, gastrointestinal tract, lungs, and heart. This fibrosis process is mostly caused by connective tissue fibroblasts and myofibroblast cells, which are influenced by various growth factors and activate the Ras signaling pathway to produce extracellular matrixes and collagen. Salirasib is a small molecule containing farnesyl isoprenoid, which is considered a Ras inhibitor. In this study, we investigated the role of the Ras protein inhibitor, salirasib, in the inhibition of genes involved in the fibrosis process in SSc skin fibroblasts in the presence of TGF-β (as an activating factor for fibroblasts).

Methods: Skin biopsies were obtained from 10 patients with SSc. Skin Fibroblasts were cultured and confirmed using immunocytochemistry (ICC). Fibroblast cells were treated with TGF-β and a Ras inhibitor (Salirasib). The gene expression level involved in the fibrosis process (e.g. COL1A1, COL1A2, CTGF, FN1, ACTA2, and TGFB1) were quantified using Real-time PCR.

Results: Our data indicated that COLA1 and CTGF mRNA levels were significantly upregulated by TGF-β. However, salirasib downregulated the expression of genes involved in fibrosis in a time- and dose-dependent manner and significantly decreased the expression of COLA1, COLA2, FN1, CTGF, TGF-β, and ACTA2 compared to TGF-β-treated group. Salirasib also significantly increased gene expression of MMP1 (matrix metalloproteinases1) than TGF-β-treated groups.

Conclusion: Based on the effects of salirasib on the expression of the genes involved in the fibrosis process; it can be considered as a new therapeutic strategy for SSc.

Keywords: Fibroblast, Fibrosis, Salirasib, Systemic sclerosis

Human Fibroblast-Like Synoviocyte Isolation Matter: A Comparison between Cell Isolation from Synovial Tissue and Synovial Fluid from Patients with Rheumatoid Arthritis

Parisa Zafari[1], Alireza Rafiei[1], Fatemeh Faramarzi[1], Mahdi Taghadosi[2]

1. Mazandaran University of medical sciences, Sari, Iran.
4. Kermanshah University of medical sciences, Kermanshah, Iran.

Background: Cell culture technology has become a popular method in the field of cell biology, pharmacology, and medical researches. Primary cells represent the normal physiological condition of human cells. Fibroblasts are the most common native cells of connective tissue that play a crucial role in the entire pathogenesis of various disorders, such as rheumatoid arthritis (RA). Fibroblast-like synoviocytes (FLSs), which overlie the loose connective tissue of the synovial sublining, are known to be the central mediators of joint damage. The mostroutine approach for the isolation of FLS is an enzymatic digestion of synovial tissue. This experimental study is designed to introducean easy, fast, and high-throughput method compared with enzymatic digestion for isolation of FLS.

Methods: The synovial tissue and synovial fluid (SF) samples were collected from eight patients with RA who underwent routine knee replacement surgery. Synovial tissue was incubated with collagenase VIII enzyme, while SF was washed with a similar volume of phosphate buffered saline. The cells were further subcultured and stored based on the standard protocols. The purity of isolated synoviocytes was confirmed using flow cytometry analysis.

Results: Isolation of FLS from SF was more successful with a faster rate, 3–5 days after culture. The morphological assessment and flow cytometry analysis confirmed the purity of SF-derived cells in passage 4.

Conclusion: SF could be a more accessible source of FLS than synovial tissue. Obtaining primary FLS from SF is a simple, fast, and cost-effective way to have a large-scale cell during a short time.

Keywords: Cell cuCell isolation, Fibroblast-like synoviocyte, Synovium, Synovial fluid

Induced Pluripotent Stem Cells Modulate the Wnt Pathway in the Course of the Bleomycin-Induced Model of Idiopathic Pulmonary Fibrosis

Paria Bayati[1], Marjan Taherian[1], Hadi Poormoghim[2], Nazanin Mojtabavi[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Rheumatology, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran

Background: The Wnt signaling pathway is a major contributor to the pathogenesis of several inflammatory disorders as well as malignancies. It has been demonstrated that the components of the pathway are aberrantly expressed in the course of fibrotic disorders too, hence we aimed to assess the potential of the induced pluripotent stem cells in modulating the expression of the cardinal genes of the Wnt pathway in a mouse model of idiopathic pulmonary fibrosis.

Methods: C57Bl/6 mice were randomly divided into three groups of Control, BLM, and BLM+IPS; the BLM mice received intratracheal instillation of bleomycin, BLM+IPS mice received tail vein injection of IPS cells 48 hours post instillation of the BLM; The Control group received PBS instead. After 3 weeks, the mice were sacrificed and Histologic assessments including hydroxy proline assay, H&E, and Masson trichrome staining were performed. The expression of the genes for Wnt, β-Cattenin, LEF, and DKK1 was assessed utilizing specific primers and SYBR green master mix. Data were analyzed and plotted utilizing GraphPad PRISM software. ANOVA test was used for comparing the results.

Results: Histologic assessment revealed successful development of the IPF model, also it was observed that the inflammation and fibrotic lesions were significantly alleviated in the BLM+IPS group. Besides, the gene expression analyses demonstrated the upregulation of Wnt, β-Cattenin, and LEF, along with the downregulation of the DKK1 gene which regulates the activation of the Wnt pathway; subsequently it was found that the treatment of the IPF mice with IPS cells results in the downregulation of the Wnt, β-Cattenin, and LEF, as well as upregulation of the DKK1 ($p<0.05$).

Conclusion: The current study highlights the therapeutic effect of the IPS cells on the IPF mouse model in terms of regulating the expression of the factors contributing to the Wnt signaling pathway to a level similar to the control healthy mice. Furthermore, the efficacy of employing IPS cells in the treatment of IPF was once more confirmed which can be expanded to clinical practice as well.

Keywords: Wnt, Bleomycin, IPF, iPScs

Induced Pluripotent Stem-cells Inhibit Experimental Bleomycin-induced Pulmonary Fibrosis through Regulation of the Insulin-like Growth Factor Signaling

Paria Bayati[1], Marjan Taherian[1], Hadi Poormoghim[2], Nazanin Mojtabavi[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran; Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran Universityof Medical Sciences, Tehran, Iran
2. Firoozgar Hospital, Iran University Of Medical Sciences, Tehran, Iran.

Background: Idiopathic pulmonary fibrosis (IPF) is among the illnesses with a high mortality rate, yet no specific cause has been identified; as a result, successful treatment has not been achieved. Among the novel approaches for treating such hard-to-cure diseases are induced pluripotent stem cells (IPSCs). Some studies have shown these cells' potential in treating IPF. Therefore, we aimed to investigate the impact of IPSCs on insulin-like growth factor (Igf) signaling as a major contributor to IPF pathogenesis.

Methods: C57BL/6 mice were intratracheally instilled with Bleomycin (BLM) or phosphate-buffered saline; the next day, half of the bleomycin group received IPSCs through tail vein injection. Hydroxyproline assay and histologic examinations have been performed to assess lung fibrosis. The gene expression was evaluated using specific primers for Igf-1, Igf-2, and insulin receptor substrate 1 (Irs-1) genes and SYBR green qPCR master mix. The data have been analyzed using the 2-ΔΔCT method.

Results: The mice that received Bleomycin showed histological characteristics of the fibrotic lung injury, which was significantly ameliorated after treatment with IPSCs comparable to the control group. Furthermore, gene expression analyses revealed that in the BLM group, Igf1, Igf2, and Irs1 genes were significantly upregulated, which were returned to near-normal levels after treatment with IPSCs.

Conclusion: IPSCs could modulate the bleomycin-induced upregulation of Igf1, Igf2, and Irs1 genes. This finding reveals a new aspect of the therapeutic impact of the IPSCs on IPF, which could be translated into other fibrotic disorders.

Keywords: iPSCs, IPF, IGF1, IGF2

Interleukin-2 therapy in Rheumatoid Arthritis patients: Systematic Review of Clinical Trials

Hale Radi[1], Gholam Ali Kardar[2], Elaheh Ferdosi-Shahandashti[1,3]

1. Department of Medical Microbiology and Biotechnology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.
2. Immunology, Asthma & Allergy Research Institute, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran.
3 Biomedical and Microbial Advanced Technologies (BMAT) Research Center, Health Research Institute, Babol University of Medical Sciences, Babol. IR.Iran.

Background: The use of cytokines such as interleukin 2, which by stimulating the proliferation of effector and regulatory T cells, stimulate or regulate immune system responses, is one of the new immunotherapy strategies for cancer and autoimmune patients. Interleukin 2 has an essential role in the proliferation of T cells in a paradoxical way, so that the use of its low dose is used for the proliferation of regulatory T cells and the use of its high dose is used for the proliferation of effector T cells. This characteristic of interleukin 2 makes this glycoprotein used in the treatment of a wide range of diseases, including rheumatoid arthritis.

Methods: PubMed, Scopus and Cochrane Library databases were searched from 2016 to 2023 for English clinical trials studies of low-dose Interleukin-2 therapy in Systemic Lupus Erythematosus patients. Titles and Abstracts were screened for suitable using predetermined inclusion and exclusion criteria.

Results: Interleukin-2 receptors on T cells are dose dependent. Low-dose IL-2 selectively adjusts FOXP3+ regulatory T cells and reductions of inflammatory cytokines to treat rheumatoid arthritis by suppressing immune responses. There were no drug-related serious adverse events (SAEs). Adverse events such as transient fever, injection-site reactions without any medical intervention were observed.

Conclusion: The results of the effectiveness of interleukin 2, which were reviewed in this systematic review article, can pave the way for more clinical studies on a wide range of autoimmune diseases.

Keywords: Interleukin-2, Rheumatoid Arthritis, Immunotherapy, Regulatory T cells

Investigation of Prognostic Markers Genes expression (miR-16, miR-132) in Follow-up and Treatment Process of Rheumatoid Aarthritis Patients in Ilam

Morteza Hosseinzadeh[1], Sahand Karami[1], Nasrin Bazgir[2], Peyman Safarzadeh[1], Saeideh Hatami[3], Hojat Sayadi[4]

1. Department of Immunology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran.
2. Department of Rheumatology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran.
3. Department of Tissue Engineering and Regenerative Medicine, Iran university of Medical Sciences, Tehran, Iran.
4. Department of Biostatistics, School of Health, Ilam University of Medical Sciences, Ilam, Iran.

Background: Rheumatoid arthritis is a chronic inflammatory disease with idiopathic causes characterized by peripheral polyarthritis. This disease is the most common form of chronic joint inflammation and often causes joint damage and physical disability its main target tissues are the synovial covering of joints, bursa, and tendon sheath. This disease is an autoimmune disease with unknown causes, and one of the reasons is the role of miRNAs in the disease progression. In this study, the expression levels of miRNA-16 and miRNA-132 and their relationship with clinical status in rheumatoid arthritis patients were investigated.

Methods: All patients with rheumatoid arthritis referring to the rheumatology subspecialty office in Ilam city and in equal numbers with appropriate age and gender are healthy control samples. In order to select samples of the patient group, 36 patients who are known to be RA patients based on clinical diagnostic tests were selected. To select healthy control subjects, simple random selection was made from healthy subjects who were diagnosed as healthy based on the opinion of experts and had no family history of RA disease. Patients were sampled before and 4-5 months after treatment then by RNA Extraction and DNA synthesis, miR-16 and miR-132 gene expression was performed by real-time PCR method.

Results: miR-16 gene expression significantly decreased after treatment of rheumatoid arthritis patients compared to before treatment, (p=0.0435), But this finding was not observed in miR-132. Also, miR-16 gene expression in rheumatoid arthritis patients with severe symptoms compared to mild symptoms was investigated before and after treatment and it was observed that there was a significant increase before treatment (p=0.005), and a significant decrease after treatment (p<0.0001), but this issue is about miR-132 was not observed. Also, the comparison of RF, CRP, and Anti-CCP tests in the serum of rheumatoid arthritis patients, before and after treatment, shows that all values show a significant decrease after treatment. p= (0.001), (0.017), and (0.001).

Conclusion: In this study, it was found that the expression of miR-16 and miR-132 increased with rheumatoid arthritis, although the expression of miR-16 shows a greater correlation with the patient's clinical symptoms, and this increase in gene expression is much greater in patients with severe symptoms and also after 4-5 months of treatment, the expression of these genes decreased, and therefore, they can be used as disease follow-up markers to check the response to treatment.

Keywords: Rheumatoid arthritis, miRNA-16, miRNA-132

Investigation the Frequencies of TCD8+ Memory Cell Subsets in Pregnant Women Complicated with Lupus

Andisheh Mosaffa Jahromi[1], Leila Bordbar[1], Niloofar Namazi[2], Bahia Namavar Jahromi[3], Moslem Ahmadi[1], Behrouz Gharesi-Fard[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Gynecology and Obstetrics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease that predominantly affects women of childbearing ages. Pregnant women with SLE are at greater risk of adverse pregnancy outcomes. Memory T-cells play role in pathogenesis of both SLE and pregnancy complications. No study has been conducted on the frequency of CD8+ memory T cells in women who simultaneously complicated with lupus and pregnancy. Therefore the aim of the present study was investigating the frequencies of TCD8+ memory cell subsets (TSCM, TCM, TEM, TEMRA, TFHM) in the peripheral blood of pregnant women with SLE and their correlation with pregnancy complications.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from the peripheral blood of 28 pregnant women with SLE with mild and severe pregnancy complications, and 15 healthy pregnant women. Four-color flow cytometry technique was used to detect the frequencies of TCD8+ memory cell subsets.

Results: The frequency of TSCMCD8+ cells was higher in pregnant women with SLE (p=0.02). Besides, a significant increase in the frequency of TCMCD8+ cells in patients with severe pregnancy outcome compared to those with mild pregnancy complications and also control group was observed (p=0.002 and 0.02, respectively).

Conclusion: In conclusion the results of the present study suggested that in the case of lupus, disruption in the balance of TCD8+ memory cells might be important in pregnancy outcome.

Keywords: Memory T cell subsets, Lupus, Pregnancy complications

Peripheral Distributions of IL-4-Producing CD4 + T Cells and CD4 + CD25 + Foxp3 + T Cells (Tregs) in Rheumatoid Arthritis Patients with Poor Response to Therapy are Associated with ACPA Status

Ashkan Rasouli-Saravani[1], Ghasem Solgi[2]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

Background: Specific profiling of CD4 + T cell subsets in the circulation and inflamed joints of rheumatoid arthritis (RA) patients may have therapeutic implications.

Methods: This study aimed to evaluate the peripheral distributions of Th2 and Treg cells in relation to anti-cyclic citrullinated peptide antibody (ACPAs) status in patients with good response (GR) and poor response (PR) to treatment. The frequencies of IL-4-producing CD4 + T cells (Th2) and CD4 + CD25 + Foxp3 + T cells (Tregs) were determined by flow cytometry in 167 RA patients including 114 GR and 53 PR cases. CD4 + T cell subsets were also analyzed based on ACPAs statuses.

Results: 63.4% of 167 patients were positive for ACPAs. Higher frequencies of Th2 ($P = 0.001$) and Treg cells ($P = 0.03$) were found in the patients versus controls. Increased and decreased frequencies of Th2 and Tregs cells were observed in the PR versus GR patients, respectively ($p = 0.003$ and $p = 0.004$). Higher proportions of Th2 cells were observed in ACPA+RA versus ACPA−RA ($p = 0.005$). Treg cells frequencies decreased in ACPA+RA versus ACPA−RA ($p = 0.02$). ACPA+GR patients showed higher proportions of Th2 cells than ACPA−GR patients ($p = 0.02$ and $p = 0.01$).

Conclusion: Analysis of the CD4 + T cell subsets profiles in conjunction with autoantibodies patterns can be useful for precise therapeutic response monitoring in the RA patients.

Keywords: Rheumatoid arthritis, ACPA, Th2, Treg

Survivin; a Novel Therapeutic Target that Correlates with Survival of Autoreactive T Lymphocytes Obtained from Patients with Ankylosing Spondylitis

Navid Shomali[1], Siamak Sandoghchian[1]

1. Tabriz University of Medical Science, Tabriz, Iran.

Background: Ankylosing spondylitis (AS) is progressive immune-mediated arthritis. Persistent autoreactivity of T cells with an up-regulated Survivin expression is strongly implicated in AS immunopathogenesis. Besides, Survivin can inhibit proapoptotic caspase 9 activations. Moreover, microRNAs are small non-coding RNAs that are dysregulated in various diseases, in which their altered expression could modulate Survivin expression. The primary goal of this study was to assess the role of Survivin and its-targeting microRNAs in the immunopathogenesis of AS disease.

Methods: For this aim, peripheral blood mononuclear cells (PBMCs) were isolated from 15 patients with AS and healthy matched controls using Ficoll-Hypaque. T cells were obtained using the magnetic-activated cell sorting (MACS) method. After that, the expression levels of Survivin, Caspase 9, and specific miRNAs were determined using qT-qPCR. Also, the expression of Survivin and Caspase 9 at protein levels was determined by western blotting. Then, the isolated T cells were co-cultured with interleukin (IL)-2 and muromonab-CD3 (OKT-3) for active-induced cell death (AICD) induction, Survivin siRNA for inhibition of Survivin expression, and their combination to assess the implication of Survivin expression in autoreactive T lymphocytes' resistance to apoptosis by determining the rate of apoptosis by Flowcytometry assay.

Results: The results showed that Survivin was up-regulated while Caspase 9 was downregulated in patients with AS. It was also revealed that microRNAs that directly or indirectly target the Survivin mRNA were dysregulated in patients with AS. It was also revealed that T cells obtained from AS patients were more resistant to apoptosis induction than those obtained from healthy people.

Conclusion: In summary, the results obtained from this study showed that dysregulation of Survivin and Survivin-targeting miRNAs in T lymphocytes obtained from AS patients contribute to their resistance to apoptosis, suggesting the future development of targeted therapies for AS.

Keywords: Ankylosing spondylitis, Apoptosis, Autoreactive T cells, Caspase 9, Survivin, miRNA

The Impacts of Exosomes Derived from PBMCs of Ankylosing Spondylitis Patients on T cell Profiles

Sina Baharaghdam[1], Mehdi Yousefi[1]

1. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Ankylosing spondylitis (AS) is one of the progressive inflammatory diseases whose important complication is affecting the articulation of the axial skeleton. Investigations in this field show that T lymphocytes are an effective factor in the pathogenesis of AS. In this study, we evaluated the role of PBMC exosomes from AS patients on the normal T cells function and expression profile.

Methods: Blood was drawn from individuals and exosomes were isolated from PBMCs. In order to evaluate the expression, secretion of different cytokines, and concentration of transcription factors of T cells, these cells were cultured and treated with PBMC exosomes.

Results: Data demonstrated an increased expression of RORγt (1.232±0.3527 fold versus 0.9220±0.1727) STAT3 (1.242±0.5935 fold versus 0.9052±0.2497) and T-bet (1.230±0.4558 fold versus 0.8580±0.3406) in the group that treated with PBMC exosomes derived from AS compared to PBMC exosomes obtained from healthy individuals. Furthermore, the concentration and expression levels of cytokines such as IL-17, IL-23, TNF-α, and IFN-γ were significantly increased in group that treated with PBMC exosomes from patients with AS. The expression level of FOXP3 (0.7092±0.1902 fold versus 1.158 ±0.6027), TGF-β (0.8236±0.2831 fold versus 1.166±0.5085) and IL-10 (0.7584±0.1989 fold versus 1.209±0.5317) were significantly decreased in the group that treated with PBMC exosomes from AS patients in comparison with the healthy PBMC exosomes group, respectively.

Conclusion: PBMC exosomes obtained from patients with AS could modify T cell profile and induce normal T cells into an inflammatory situation by transcription factors derived from Th1 and Th17 and upregulation of cytokines, as well as downregulation of Treg transcription factors and cytokines.

Keywords: Ankylosing spondylitis, Exosomes, Th17, Th1, Treg

The Suppression of IL17/IL23 Immunopathogenic Axis by Curcumin-Niosome in Skin Lesions of Psoriatic Patients: A Double-Blind Placebo-Control Clinical Trial Study

Hanieh Kolahdooz[1], Vahid khori[2], Vahid Erfani moghadam[3], Fatemeh Livani[4], Saeed Mohammadi[5], Ali Memarian[6]

1. Student Research Committee, Department of Medical Immunology, Golestan University of Medical Sciences, Gorgan, Iran.
2. Ischemic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran.
3. Department of Medical Biotechnology, Faculty of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran.
4. Clinical Research Development Unit (CRDU), Sayad Shirazi Hospital, Golestan University of Medical Sciences, Gorgan, Iran.
5. Stem Cell Research center, Golestan University of Medical Sciences, Gorgan, Iran.
6. Department of Immunology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran.

Background: Psoriasis (PS) characterize by epidermal hyperplasia and dermal infiltration of immune cells. An inconsequential capability of percutaneous penetration for most topical anti-inflammatory drugs is one of the main causes of therapeutic failure. Although curcumin has indicated efficacy in regulating the gene expression of inflammatory cytokines such as IL-17, IL-23, IL-22, TNFα, etc…., its effective penetration through the stratum corneum is still a challenging issue during transdermal transfer. Therefore, niosome nanoparticles were used as curcumin carriers to increase its delivery and anti-inflammatory effects in skin lesions. Materials and

Methods: This was a randomized, double-blind, placebo-controlled study. Patients were stratified according to age (<65 or ≥18 years) and Psoriasis Area and Severity Index (PASI score) <30 and randomly assigned to receive curcumin-niosom (CUR-NIO) and the placebo. CUR-NIO was prepared by the thin-film hydration method. This suspension was sonicated, filtered, and lyophilized. Hyaluronic acid and collagen were slowly added into the lyophilized CUR-NIO. Two similar-parallel lesions of PS patients were selected and instructed to apply the 0.1% CUR-NIO gel versus placebo twice a day for 4 weeks on their eligible skin lesions. Skin punches were obtained and Real-time q-PCR amplifications were performed.

Results: As evaluated, employing CUR-NIO gel had efficacious results on the patients' lesions. There was a significant reduction in redness, itching, scaling, and an apparent improvement in CUR-NIO-treated compared to the placebo-treated counterpart lesions. Moreover, the gene expression analyses also showed significantly decreased (P<0.05) levels of IL17, IL23, IL22, TNFα, S100A7, S100A12, and Ki67 in CUR-NIO-treated lesions.

Conclusion: According to our study, the administration of our CUR-NIO gel could stimulate therapeutic effects on the psoriatic skin lesions and lead to healing processes. This could be induced by expression regulation in the main pathogenic inflammatory genes in PS patients. CUR-NIO gel possibly will be a promising drug for the improvement of the life quality of PS patients.

Keywords: Curcumin, IL17, IL23, IL22, Ki67, Niosome, Psoriasis, S100A7, S100A12, TNFα

Immunoparasitology

Attenuation of immune system following administration of *Fasciola hepatica* total extract and fatty acid binding protein in experimental autoimmune encephalomyelitis.

Maryam Hajizadeh[1], Ahmad Reza Meamar[1], Reza Falak[2], Ali Akbar Saboor-Yaraghi[3]

1. Department of Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences (IUMS), Tehran, Iran
2. Immunology Research center (IRC), Iran University of Medical Sciences, and Tehran, Iran.
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: An increasing body of previous studies have shown that *Fasciola hepatica* plays play an important role in the development of the immune system. The aim of the present study was to explore whether the fatty acid binding protein (FABP) extracted from *Fasciola hepatica* can affect in the function of immune system in a mouse model of experimental autoimmune encephalomyelitis (EAE).

Methods: EAE-induced C57BL6 mice were treated with vehicle, parasite total extract (TE) or FABP. The clinical signs, body weights, and the expressions of IFN-γ, T-bet, IL-4, GATA3, IL-17, RORγ, TGF-β FOXP3, IL-10 and TNF-αgenes and proteins were determined in the isolated CD4+ splenocytes. Besides, the percentage of Tregs and the degree of demyelination were evaluated.

Results: We found that TE and FABP treatments decreased the clinical scores, lymphocyte infiltration rate, and demyelinated plaques in EAE mice. The expressions IL-4 and GATA3 were increased, whereasIL-17 and TNF-αwere down-regulated. FABP didn't affect the expression of IFN-γ, RORγ and TGF-βgenes and proteins but reduced the expression of IL-10 and T-bet. TE administration didn't affect the expression of Tbet and IL-10 genes, but increased expressions of IFN-γand FOXP3 in CD4+ lymphocytes.

Discussions: The present study indicates that *F. hepatica* FABP and TE were both capable to suppress the inflammatory responses in EAE mice and could shift the immune system toward Th2 responses. However, FABP exerts stronger anti-inflammatory effects and seems to be more effective than TE in EAE mice.

Keywords: *Fasciola hepatica*, multiple sclerosis, FABP, EAE, helminth therapy

Case Report: Challenges for the Diagnosis and Treatment of *Strongyloides stercoralis* in Chronic Obstructive Pulmonary Disease Patients

Aliraza Ashiri[1], Molouk Beiromvand[2], Abdollah Rafiei[2], Abdollah Khanzadeh[3], Arash Alghasi[4]

1. Department of Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran;
2. Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. Abadan School of Medical Sciences, Abadan, Iran.
4. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: Strongyloidiasis, a neglected tropical disease (NTD), which is caused by *Strongyloides stercoralis*, can be fatal in immunocompromised patients. In most chronic cases, infections most frequently are asymptomatic, and eosinophilia might be the only clinical characteristic of this disease. The use of corticosteroids in some diseases like chronic obstructive pulmonary disease (COPD) may lead to the development of the life-threatening *S. stercoralis* hyperinfection syndrome.

Case presention: In the present research, we presented five cases of strongyloidiasis with a history of COPD and receiving corticosteroids from Abadan County, southwestern Iran. By performing the direct smear stool examinations, two cases were identified and the other three cases were diagnosed using the agar plate culture method. Despite reporting eosinophilia in previous patients' hospitalizations, the fecal examination was not performed for parasitic infections. Moreover, pulmonary symptoms were similar, but gastrointestinal symptoms were varied, including nausea, vomiting, abdominal pain, epigastric pain, constipation, and diarrhea. All the included patients were treated with albendazole, which is the second-line drug for *S. stercoralis*, and relapse of infection was observed in two patients by passing few months from the treatment. The increased blood eosinophil count was shown to play important roles in both the management of COPD and diagnosis of helminthic infections.

Conclusion: In COPD patients who are receiving steroids, screening and follow-up for strongyloidiasis should be considered as priorities. In addition, ivermectin, which is the first-line drug for strongyloidiasis, should be available in the region.

Keywords: Strongyloidiasis, COPD, Eosinophilia, Corticosteroid.

Comparison of the immune responses of macrophages of BALB/c and C57BL/6 mice exposed to saponin adjuvant and *Leishmania major* parasite lysate

Mahshid Mohatshamifard[1], Ahmad Zavaran Hosseini[1], Sara Soudi[1]

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background: Leishmania is an intracellular parasite Although Leishmania is not associated with high mortality, the high infection rate, and deformed skin lesions allow it to be categorized as a type 1 (untreated and uncontrolled) infection. Given that current treatment approaches have significant toxicity and side effects and reported drug resistance, cell therapy is one of the new treatment methods that has piqued the interest of researchers. Because infected macrophages (MQs) are the primary site of parasite multiplication, and macrophages, with their dual activity (inflammatory and anti-inflammatory), play an important role in disease progression, macrophages are one of the preferred candidates for leishmaniasis cell therapy. To better understand macrophage immune responses, researchers studied macrophages from two mouse breeds, BALB/c (parasite-sensitive animals) and C57BL/6 (resistant mice), in the presence of *Leishmania major* parasite (L. major) and adjuvant saponin and parasite lysates. Additionally, the polarization of macrophages from the M2 to the M1 phenotype in the presence of saponin adjuvant and parasite lysate is investigated. In this study we assess the functional plasticity and polarization of macrophages after exposure to saponin adjuvant and *Leishmania major* lysate, as well as to compare macrophages in BALB/c mice and C57BL/6 mice. Also, the polarization of macrophages from M2 to M1 phenotype was investigated.

Methods: Macrophages isolated from the peritoneum of mice were cultured in the presence of the Leishmania parasite, saponin adjuvant, and parasite lysate. Inflammatory and anti-inflammatory cytokine production, nitric oxide (NO) production, the number of infected cells, and the parasite burden of macrophages were all evaluated using the supernatant of these cells. We determined the required dose of protein antigens (parasite lysate) by the Bradford test, cytokines were analyzed using the ELISA test and the amount of nitric oxide produced was measured by the nitric oxide assay (Griess method).

Results: Macrophages from both groups showed an increased level of nitric oxide (NO) production and inflammatory cytokines like TNF-α and IL-6. It also showed a decreased level of anti-inflammatory cytokines such as TGF-βand IL-10. It should be mentioned that this increase and decrease were more in C57BL/6 (resistant mice). Furthermore, the number of infected cells and the parasite burden of macrophages in the test groups were significantly reduced compared to the controls.

Conclusion: These findings show that the ability of saponin adjuvants and *Leishmania major* parasite lysate to repolarize these macrophages to M1 suggests that moving from M2 to M1 phenotype may provide a therapeutic possibility worthy of future pharmacological research.

Keywords: Macrophage, Leishmania lysate, Immune response, cytokines, saponin

Cross-reactive immune responses between apyrases from *Phlebotomus kandelakii*, and *Phlebotomus Papatasi*

Soheila Ajdary[1], Shima Fayaz[2], Fariborz Bahrami[1], Parviz Parvizi[3], Pezhman Fard-Esfahani[2]

1. Department of Immunology, Pasteur Institute of Iran, Tehran, Iran.
2. Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran
3. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran

Background: Leishmania (L.) parasites are inoculated into the host skin along with sand fly saliva which consists of anticoagulant, anti-inflammatory, and immunomodulatory molecules. Saliva plays a crucial role in the establishment of Leishmania infection in vertebrate hosts. The composition and immunogenicity of the salivary proteins are mostly species-specific; however, few studies have reported cross-reactivity between closely-related species. In Iran, *Phlebotomus (P.) kandelakii*, and *P. Papatasi* are the prevalent vectors of visceral and cutaneous leishmaniasis, respectively. In the present study, the immunogenicity and potential cross-reactivity of apyrase- one of the prominent proteins of sand flies' saliva- from *P. kandelakii* (Pkapy) and *P. Papatasi* (Ppapy) were investigated.

Methods: Pkapy and Ppapy were expressed in *Escherichia (E.) coli* BL21. BALB/c mice were immunized with the proteins along with Quil A, as an adjuvant. Humoral responses were evaluated by ELISA and dot-blot analysis. T cell proliferation was assessed using Alamar blue.

Results: The results indicated that ~39 kDa recombinant apyrases were successfully expressed in E. coli. Both Pkapy and Ppapy induced significant levels of antibodies against respective recombinant proteins, and cross-reactivity between them was documented. Moreover, T cell proliferation assay indicated that the immunized mice could specifically recall responses to their homologous proteins. In addition, cross-reactive T-cell responses was documented between Ppapy and Pkapy.

Conclusion: The recombinant Pkapy and Ppapy are immunogenic in BALB/c mice. The cross-reactive function of these recombinant proteins suggests a potential role for apyrase as a cross-exposure marker and even as a cross-reactive anti-Leishmania vaccine in the future. Further studies are required to verify the protective responses of these proteins, and to confirm the cross-reactive responses in endemic areas of leishmaniasis.

Keywords: Leishmania, sand fly, Apyrase, immune response

Design of a new electrochemical immunosensor based on screen printed carbon electrode modified with carbon nanofibers and gold nanoparticles for detecting anti-*Toxoplasma gondii* IgG antibodies

Mahboobeh Salimi[1], Hossein Keshavarz Valian[1], Mahdi Mohebali[1], Mahdi Adabi[2], Saeedeh Shojaee[1]

1. Department of Medical Parasitology and Mycology, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Medical Nanotechnology, Tehran University of Medical Sciences, Tehran, Iran

Background: Serologic detection of anti-*Toxoplasma gondii* antibodies (anti-*T.gondii*) plays a key role in clinical diagnosis of human toxoplasmosis. This study describes the development of a new electrochemical immunosensor for detecting anti-*T.gondii* IgG in human sera based on carbon nanofibers (CNFs) and gold nanoparticles (AuNPs).

Methods: CNFs was produced using electrospinning and carbonization processes. Screen printed carbon electrode (SPCE) surface modified with CNFs and gold nanoparticles electrodeposited onto the CNFs. *T.gondii* antigens was immobilized onto the CNFs-AuNPs/SPCE. Serum samples, including anti-*T.gondii* IgG, were coated on the modified electrode, and then assessed via adding anti-human IgG labeled with horseradish peroxidase (HRP). The morphology of CNFs, and CNFs-AuNPs/SPCE surface were characterized using field emission scanning electron microscope (FESEM). Structures of CNFs were evaluated by Raman spectroscopy and X-ray diffraction (XRD). Electrochemical assessment of anti-*T. Gondii* IgG was carried out using differential pulse voltammetry (DPV).

Results: Under optimal conditions, the immunosensor could detect anti-*T.gondii* IgG in the range of 0–200 U ml-1, with a limit of detection of 9×10-3 U ml-1. The proposed immunosensor was exhibited high selectivity, strong stability, and acceptable reproducibility and repeatability. Furthermore, there was a strong correlation between results obtained via the designed immunosensor and standard enzyme-linked immunosorbent assay (ELISA) method.

Conclusion: The developed immunosensor was demonstrated as a promising method for the rapid and accurate clinical diagnosis of toxoplasmosis.

Keywords: Electrochemical immunosensor, Anti-*Toxoplasma gondii* IgG, Carbon nanofibers, Gold nanoparticles

Effect of *Arnebia euchroma* on immune response of BALB/c mice infected with Leishmania major

Rasool Rahimi Junqani[1], Mohammadreza Alijani[1], Iraj Mirzaee Dizgah[2], Saeed Habibian Dehkordi[3]

1. Graduated Student of Veterinary Medicine, Shahrekord University, and Shahrekord, Iran.
2. Department of Basic Sciences, School of Medicine, Artesh University of Medical sciences, Tehran, Iran
3. Department of Pharmacology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Background: *Leishmania major* is an important intracellular parasite that causes cutaneous leishmaniasis. A protective immune response to this parasite requires the development of a Th1 CD4+ T cell phenotype. The present study was aimed to evaluate the effect of *Arnebia euchroma* on immune response of BALB/c mice infected with Leishmania major.

Methods: A total of 30 BALB/ c mice were randomly divided into three groups including control (cutaneous leishmaniasis mice without any treatment), group 1 (cutaneous leishmaniasis mice received Vaseline) and group 2 groups (cutaneous leishmaniasis mice treated). Mice were challenged with 2×106 promastigote subcutaneously and the treatment was performed 3 times daily for 30 days. On days 7, 14, 21 and 28 post-treatment wound diameter was measured. At the end of the treatment the serum levels of CD4+, CD8+ lymphocytes and CD4/CD8 ratio were determined using flowcytometry method.

Results: The group treated with *Arnebia euchroma* showed significantly deceased wound diameters on the days 14 and 21 post-treatment compared to both the groups 1 and 2. In addition, the findings of the present study showed an increase in the serum levels of CD4+ lymphocytes in the treatment group, and as a result the ratio of CD4/CD8 was significantly high in this group.

Conclusion: Our data indicate that *Arnebia euchroma* treatment enhances the immune response in BALB/c mice against L. major infection and raises the possibility of utilizing this approach for treatment strategies.

Keywords: *Leishmania major, Arnebia euchroma*, Immune evaluation

Effect of *Fasciola hepatica* protein products on Asthma and Allergy

Kobra Mokhtarian[1], Reza Falak[2], Hassan Khadempour[2]

1. Cellular & Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
2. Immunology & Infectious Disease Research Institute Iran University of Medical Sciences

Background: Asthma is a common chronic respiratory disease that subsequently leads to increased contractility of the smooth muscle. The modern medical system has not yet found a definitive treatment for asthma. Protein products, special cysteine proteases derived from *Fasciola hepatica*, play an important role in facilitating biological functions such as immune system invasion and tissue invasion. Today, these proteins are used in new immunotherapies, diagnostics, and antiparasitic drugs. Therefore, the aim of this study was to investigate the effect of cysteine proteases of *Fasciola hepatica* on the allergy model in Balb/c mice.

Methods: The female BALB/C mice (6-8 weeks old; n=8) were purchased from the Pasteur Institute. They were housed in standard conditions. All animal procedures were approved by the Ethics Committee of Shahrekord University of Medical Science (IR.SKUMS.REC.1397.4). This study was conducted based on the standard protocol of OVA-albumin to induce experimental allergic asthma in a mouse model. Mice in the model group were given an intraperitoneal injection of 0.5 mL OVA (Sigma, Germany, 2 mg/mL) on days 1, 7, and 14 for sensitization. And From day 17, mice in the model group were given aerosol inhalation of 1% OVA for excitation 3 times a week, 30 min each time. Mice in the Control group were challenged with the same amount of PBS. After anesthesia, the mice were sacrificed, and the blood was collected. Some important cytokines such as IL-4, IL-10, INF-γ, and TGF-βwere measured by ELISA methods.

Results: The results showed that Il-10 and INF- γin the control, allergy, prophylaxis, and treatment groups was a significant relation between the groups. The Il-4 and TGF-βwere changed but was no significant relation between the groups.

Conclusion: These proteins were able to regulate the immune system, it is suggested that the administrative authorities provide research on animal models and clinical trials for researchers and use of biological products for treatment.

Keywords: *Fasciola hepatica*; Proteins; Cytokines

Efficacy of mesenchymal stem cell therapy in solving parasitic drug resistance: a systematic review

Ashkan Hajjafari[1], Soheil Sadr[2], Pouria Ahmadi Simab[3], Hassan Borji[4]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University, Science and Research Branch, Tehran, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Pathobiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
4. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Nowadays, the resistance to antiparasite medicines is an alarming issue threatening animal and human lives. In both human and veterinary medicine, the over-prescription of broad-spectrum antiparasitic drugs; multiplies our need for new treatment methods. Numerous studies have demonstrated that MSC cells perform a paracrine function in tissue remodeling and controlling inflammation. The most recent pre-clinical findings in the use of MSCs as a treatment for parasitic infections were investigated in the present study.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on Anti-inflammatory and Immunomodulatory effects of Mesenchymal Stem Cell therapy on parasitic drug resistance from January 2000 to January 2023. Twenty affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: According to studies, administering MSCs to patients with parasitic infections such as schistosomiasis, malaria, cystic echinococcosis, toxoplasmosis, leishmaniasis, and trypanosomiasis decreases the parasite prevalence. MCSs modify the levels of pro- and anti-inflammatory cytokines. Additionally, administering MSCs and anti-parasitic medications improved their anti-parasitic and immunomodulatory effects. In the parasitic diseases that cause liver injuries, it is shown that BM-MSCs transplantation could be worthwhile; because these stem cells can differentiate into hepatic cells, besides the enhancement of trophic intermediaries' secretion capacity and immunomodulatory aspects.

Conclusion: In conclusion, using MSCs with anti-parasitic medications enhanced anti-parasitic and immunomodulatory actions against parasitic diseases. The affordability of stem cell-based therapies will be crucial in their uptake, especially in underdeveloped nations where these neglected tropical diseases are a significant problem because these methodologies and techniques are still in their infancy.

Keywords: Anti-inflammatory, Anti-parasite, Immunomodulatory, Mesenchymal stem cell

Evaluating immune responses to a novel multi-antigenic DNA vaccine with Calcium Phosphate nanoparticles as an adjuvant against *Toxoplasma gondii*

Rasool Rahimi Junqani[1], Mohammadreza Alijani[1], Mohammadreza Mahzonie[2]

1. Graduated Student of Veterinary Medicine, Shahrekord University, Shahrekord,, Iran.
3. Department of pathology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Background: *Toxoplasma gondii* is an important intracellular parasite which causes infections in a wide range of animals, and due to the lack of effective vaccines and drugs, remains a threatening challenge. The aim of present study was to evaluate a new multi-antigenic DNA vaccine encoding SAG1, SAG3, ROP2, GRA5, GRA7, AMA1, BAG1 and ubiquitin with co-delivery of calcium phosphate nanoparticles against *T. gondii* in mice.

Methods: Five groups of BALB/c mice were used in the present study, including three negative control groups (PBS, Pvax1 vector, and adjuvant) and two vaccination groups (PVAX1-UMAF and PVAX1-UMAF + adjuvant). The mice were immunized intraperitoneally three times (On days 0, 14 and 28) and challenged with *T. gondii* RH strain 5 weeks later. In order to evaluate tissues parasite load, three mice from each group were sacrificed after death, and their brains and spleens were removed and real-time PCR was applied for quantification of the tachyzoites in mice tissues.

Results: The mice immunized with PVAX1-UMAF and PVAX1-UMAF+adjuvant showed significantly reduced parasite load in both brains and spleens (p<0.05). In addition, the parasite load was markedly decreased by PVAX1-UMAF+adjuvant in comparison with the group immunized with PVAX1-UMAF alone, however, the significant difference was witnessed only in spleen tissue (p<0.05).

Conclusion: Our data indicate that the PVAX1-UMAF+adjuvant was effective for stimulation of immune responses and should be considered as a promising candidate in the design of new vaccines against toxoplasmosis.

Keywords: DNA vaccine, *Toxoplasma gondii*, Calcium phosphate Nano adjuvant, Immune evaluation

Evaluation of proteins involved in drug resistance of Leishmania by mitochondrial proteomics

Minoo Tasbihi[1], Faezeh Shekari[2], Homa Hajjaran[3], Ramtin Hadighi[4]

1. Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Science
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Academic Center for Education, Culture and Research, Tehran, Iran
3. Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Medical Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Antimony is an important drug for the treatment of Leishmania parasite infections. In several countries, the emergence of drug-resistant Leishmania species has reduced the effectiveness of this drug. The mechanism of clinical drug resistance is unclear. The aim of this work was to identify mitochondrial proteome alterations associated with resistance against antimonial.

Methods: A combination of cell fractionation, liquid chromatography-tandem mass spectrometry (LC-MS/MS), and Label-Free Quantification was used to characterize the mitochondrial protein composition of *Leishmania tropica* field isolates resistant and sensitive to meglumine antimoniate.

Results: LC-MS/MS analysis resulted in the identification of about 1200 proteins of the Leishmania tropica mitochondrial proteome. Various criteria were used to allocate about 40% of proteins to the mitochondrial proteome. Comparative quantitative proteomic analysis of the sensitive and the resistant strains showed proteins with differential abundance in resistance species are involved in TCA and aerobic respiration enzymes, stress proteins, lipid metabolism enzymes, and translation.

Conclusion: These results showed that the mechanism of antimony resistance in Leishmania spp. field isolate may be associated with alteration in enzymes involved in mitochondrial pathways.

Keywords: Leishmania, Drug Resistance, Mitochondrial Proteomics

Evaluation of CD4+ and CD8+ T-Cell Responses to cutaneous leishmaniasis in BALB/c mice treated with silver proteinate (Protargol) and silver sulfadiazine

Nazanin Hemmati[1], Mohammadreza Alijani[2], Rasool Rahimi Junqani[2], Saeed Habibian Dehkordi[3]

1. Student of Genetics, Shahrekord University, Shahrekord, Iran
2. Graduated Student of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
3. Department of Pharmacology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Background: Cutaneous leishmaniasis (CL) as an important public health concern is increasingly circulating by sand flies globally and effective vaccines and treatments are not currently available for this disease. The aim of present study was to evaluate the immune response against cutaneous leishmaniasis in BALB/c mice treated with silver proteinate (Protargol) and silver sulfadiazine.

Methods: In the present study, 28 BALB / c mice were at 6 to 8 weeks of age were randomly divided into four groups of 7 mice. Then, all mice were injected subcutaneously 0.1 ml of the culture medium containing 2 ×106 promastigotes. The groups include; control group (without treatment), sham group (treated with placebo), Protargol group and silver sulfadiazine group. The treatment was performed 3 times a day for 30 days in all groups. After treatment wound diameter was measured weekly and reported. Also, on week 4 blood samples were taken and serum levels of CD4+ and CD8+ lymphocytes were measured.

Results: Clinical evidences of this study revealed that the wound area in Protargol group was significantly deceased on the second to fourth weeks after treatment in comparison with control and placebo groups. Furthermore, CD4+ level was significantly higher in Protargol group compared to other groups, but no significant difference was observed among other groups. Also, the highest ratio of CD4/CD8 was in Protargol group, which was significantly higher than all other groups.

Conclusion: According to the results it can be concluded that Protargol improve the CL lesions caused by *Leishman major* in the BLAB/ c mice. In addition, Protargol by increasing the CD4+ level in serum enhance the immune system and can be considered as treatment method for Cutaneous leishmaniasis, however further investigation is required.

Keywords: Leishmania major, Protargol, Silver sulfadiazine, Immune evaluation

Evaluation of Somatic Prominent Antigens of *Toxocara canis*, and *T. Cati* Adult Helminths for Laboratory Diagnose of Human Toxocariasis Using Indirect ELISA

Zahra Navi[1], Reza Falak[2], Mehdi Mohebali[3], Mohammad Zibaei[3], Zabihollah Zarei[3], Mohammad bagher molairad[3], Mojgan Aryaipour[3], Mohammad Bagher Rokni[4], Abbas Rahimi Foroushani[4]

1. Department of parasitology and myco, Tehran, Iran.
2. Department of Immunology, School of Medicine, Iran University of Medical Sciences
3. Department of parasitology and mycology, School of public health, Tehran University of Medical Sciences
4. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences

Background: Toxocariasis accounts for soil-transmitted helminths and is listed as one of the five most important neglected tropical diseases (NTD) by CDC. Human especially children are infected by digestion of *Toxocara canis* or *T. cati* eggs. Most of diagnostic test have been designed based on excretory/secretory (ES) or recombinant proteins. In this study we tried to evaluate the somatic antigens of adult helminths for sera diagnose of this zoonotic infection.

Methods: We isolated Toxocara adult worms from 1-2 month puppies and cats naturally infected by Toxocara from Meshkin Shahr Research Center, northwestern Iran. Protein extract prepared by 0.1 molar buffer of disodium phosphate and monosodium phosphate plus added 1Mmol PMSF as anti-protease cocktail. We identified and compared adult *T. canis* and *T. cati* crude antigens through SDS-PAGE. The sensitivity and specificity of crude antigens of both species were assessed using IgG Indirect ELISA.

Results: Protein isolation of *T. canis* and *T. cati* adult worms using (SDS-PAGE) illustrated that somatic proteins of adult worm of these 2 species were almost homologous and just minor differences in the area between 15-25 kDa fractions were observed. By calculating the RF of antigenic bands molecular weights of about 10-240 kDa were evaluated. Moreover, the crude extract of adult worms presented limited capacity for detection of positive and negative serums due to the high cross-reactivity with nonspecific antibodies.

Conclusion: The sensitivity and specificity of ELISA test need improve by fractionation of crude antigens and preparing several purified antigens.

Keywords: Toxocariasis, Toxocara, ELISA

Evaluation of the effect of miR-155 - chitosan nanoparticles on *Leishmania major* infected macrophages

Azam Pourabbasi Ardekan[1], Sarvenaz Kashefi[1], Yasamin rahmani[1], Mostafa Haji Molla Hoseini[1]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences

Background: The role of miR-155 in the polarization of macrophages toward M1 has been confirmed in various studies. But there are contradictions in the results of studies regarding the function of this molecule in anti-leishmanial responses. This research investigated the effect of miR-155-producing chitosan nanoparticles on macrophages infected with *Leishmania major* (MRHO/IR/75/ER).

Methods: MiR-155 CNPs were prepared by the coacervation method, and their physicochemical properties were determined, including size, and surface charge, and also evaluated loading efficiency. The Raw 267.4 cells were treated for 48 h in 6-well plates to check transfection using fluorescent microscopy and real-time technique. The effect of miR-155 CNPs in macrophages exposed to the *Leishmania major* parasite was investigated using infectivity rate assay and Nitric oxide (NO) assay. The data are expressed as mean ± SD obtained from at least three independent experiments.

Results: Nanoparticles with size 283 nm and zeta potential +22 mV were prepared. According to the GFP expression, a significant increase in the uptake of miR-155 CNPs by Raw 264.7 cells was observed. Culture supernatant of the infected/noninfected cells gathered following 72 h and used for the measurement of nitrite. Data indicate miR-155 CNPs had a major effect on NO production by macrophages. Moreover, miR-155 CNPs decrease the infection rate of Raw 267.4 cells.

Conclusion: According to the obtained result miR-155 CNPs reduced the ex vivo parasite infectivity and this inhibitory effect related to the increased biosynthesis and release of Nitric oxide.

Keywords: Chitosan nanoparticle, MiR-155, *Leishmania major*

Evaluation the histopathological effects of *Dicrocoelium dendriticum* parasite egg on inflammatory lesions caused by ulcerative colitis in C57Bl/6 mice.

Malihe Eilakinezhad[1], Leila Mighani[1], Elham Moghaddas[1], Mehdi Zarean[1], Majid Khazaei[1], Seyed-Alireza Esmaeili[2]

1. Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Immunology Research Center, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterized by inflammation limited to the mucosal layer in the distal parts of the intestine. Colonic endothelial surface damage occurs as a result of ECM accumulation and excessive collagen deposition. Since preventing the infiltration of inflammatory cells by regulating the immune response is effective in healing mucosal injuries. Worms can modulate the immune response by shifting from TH1 to TH2 and play an effective role in preventing inflammation in autoimmune diseases including IBD. The goal of this study was to perform a comparative analysis of anti-inflammatory role of *Dicrocelium dendriticum* eggs on inflammatory lesions caused by UC in C57Bl/6 mice.

Methods: UC was induced in30male C57Bl/6 mice by DSS and confirmed by disease activity index (DAI). In the prophylaxis group, *Dicrocelium dendriticum* eggs were first injected and then the disease was induced. One treatment group give oral mesalazine after induction of the disease, and the other group was injected with *Dicrocelium dendriticum* eggs in two doses by IP. After 10 days, mice were killed and colon samples were stained with hematoxylin and eosin (H&E) and colon lesions were detected by light microscopy.

Results: Macroscopic observations show the effects of *Dicrocelium dendriticum* eggs on DAI, such as reducing colon shortening with DSS, preventing colon weight loss, and increasing spleen weight. Hematoxylin-eosin staining showed that parasite eggs decreased the histopathological score of colon tissue, which included the severity of inflammation, crypt destruction and mucosal damage. Which indicates a reduction in the symptoms of colitis in mice.

Conclusion: Considering the role of improving mucus in the pathogenesis of UC, it seems that *Dicrocoelium dendriticum* eggs play a role in improving the mucus and regulating the body's immune response in UC disease by preventing the penetration of inflammatory cells into the colon tissue.

Keywords: Immnomodulatory" inflammatory bowel disease"Inflammation

Gene Profile Expression Related to Type I Interferons in HT-29 Cells Exposed to *Cryptosporidium parvum*.

Ali Paydar[1], Seyede Manizhe Heidarnejadi[2]

1. Shoushtar University of Medical sciences & health services, Shoushtar, Iran.
2. Shoshtar Faculty of Medical Sciences, Shoshtar, IR Iran.

Background: *Cryptosporidium parvum* may contribute to upregulation or downregulation of host cellular genes among which type I (α, β) and type II (γ) interferons play key roles to eliminate infectious agents.

Methods: The current study aimed at evaluating the expressed genes related to human type I interferon response in HT-29 cell line after exposure to *C. parvum* for six and 24 hours. Methods: Subsequently, the overexpression and under expression of 84 human genes related to type I interferons were investigated using RT2Profiler™ PCR (polymerase chain reaction) Array.

Results: Four top overexpressed genes including IL10, SH2D1A, MX1, and HLA-A after six hours exposure, and 10 top overexpressed genes as IL15, DDX58, CXCL10, NMI, MYD88, STAT3, IFNAR2, IFIH1, CASP1, and TLR3 were observed after 24 hours exposure. Five underexpressed genes such as TIMP1, TYK2, IRF2, PML and IRF5 were monitored for six hours.

Conclusions: The current study findings revealed that the overexpressed genes IL15, TIMP1, and SH2D1A may have an important role to inhibit the invasion of *C. parvum*. Also, the overexpressed genes, namely SH2D1A, MX1, andNMI, may have antiviral properties while TIMP1 may have anticancer properties. Further, the pertinent results demonstrated that the type I interferons and the relevant genes had significant effects on stimulating innate immune system against *C. parvum*.

Keywords: Keywords: HT29 Cells, Type I Interferon Response, Gene Profile Expression, *Cryptosporidium parvum*, Iowa Strain.

Immune response (Th1/Th2) against Leishmania candidate vaccine (Leish-F1) in the volunteers with history of cutaneous leishmaniasis

Akram Miramin Mohammadi[1], Steeve Reed[2], Malcolm S. Duthie[3], Amir Javadi[4], Ali Khamesipour[5]

1. Center for research and training in skin diseases and leprosy, Tehran, Iran.
2. Infectious Disease Research Institute (IDRI), Tehran, Iran.

Background: Treatment of leishmaniasis is a challenging issue, especially cutaneous leishmaniasis (CL) caused by *L. tropica* and *L. braziliensis*. Currently available control measures, including environmental sanitation, drug treatments, and reservoir/vector control, are expensive and cannot be sustained effectively by poor countries due to the problems of financing and implementation. Leishmanization was shown to be effective to protect against cutaneous leishmaniasis. Efforts to search for vaccines against leishmaniasis resulted in development of several first generation crude Leishmania vaccines which were tested in phase 1 to 3 clinical trials against old and new world leishmaniasis. The results showed that the various preparations were safe and induced immune responses in humans and dogs but the efficacy data were not promising and there is still no vaccine available against any form of human leishmaniasis. A polyprotein vaccine consisting of a single fusion with multiple antigenic epitopes would be less costly to manufacture than a vaccine consisting of several recombinant proteins. For this reason, a polyprotein comprised of the three priority candidate antigens including TSA (thiol-specific antioxidant), LmSTI1 (*L. major* stress-inducible protein 1) and LeIF (Leishmania elongation initiation factor), fused in tandem, which was prepared and referred to as Leish-111f. Leish-111f (now called LEISH-F1) and LeishF2 (modified leishF1) plus IL-12 as an adjuvant, or when used as DNA plasmid, induced protection in susceptible BALB/c mice. LEISH-F1 and LeishF2 were the first 2nd generation vaccine to reach human trials and the results of a phase 2 clinical trial showed that the vaccine is safe and induced Th1 responses in volunteers with history of exposure to *L. donovani* or in volunteers with no history of exposure to Leishmania. The LEISH-F3 vaccine is a fusion polypeptide made by linking in tandem two Leishmania proteins: *L. infantum/donovani* nonspecific nucleoside hydrolase (NH) protein and L. infantum sterol 24-c-methyltransferase (SMT) protein. The purpose of this study was as follow: 1. to evaluate the immune responses generation in CL against Leishmania candidate vaccine (Leish-F2, LeishF3). 2. To compare the immune responses generation in CL against live *Leishmania major* vs. killed *Leishmania major*.

Methods: Volunteers with active CL lesion, history of CL and healthy ones were recruited. Blood samples were collected and stimulated with either live or killed L. major, SLA, recombinant antigens (individual and Fusion) and then Th1/Th2 cytokine profile was checked.

Results: IFN-γproduction in PBMC volunteers with active lesion stimulated was significantly, higher with LeishF2 (p=0.000), LeishF3 (p=0.01) than unstimulated. IFNγproduction in PBMC volunteers with history of CL stimulated was significantly higher with LeishF2 (p=0.002), LeishF3 (p<0.001) than unstimulated. There was no significant difference in IL-5 production in PBMC of healthy, active lesion, volunteers stimulated with fusion recombinant antigens and unstimulated PBMC. There was no significant different between IFN-γ levels in PBMC of healthy volunteers, active lesion, history of CL stimulated with SLA or LeishF2. There was no significant different between IL-5 Level in PBMC of healthy volunteers, active lesion, history of CL stimulated with SLA or LeishF2.

Conclusion: IFN-γlevel was similar when PBMCwas stimulated with SLA or LeishF2 but higher if stimulated with LeishF3. The Th1 response is higher in volunteers with history of CL than active lesion. The immuneresponse in CL recognized the fusion recombinant antigen in vitro.

Keywords: recombinant antigen, leishmaniasis

Investigating of phagocytosis and the expression of inflammatory genes in macrophages exposed to passage 1, *Leishmania major* in the BALB/c mice

Ehsan Mohseni[1], Hossein Rezvan[1], Sahar Hamoon Navard[1]

1. Department of Pathobiology, Faculty of veterinary Sceince, Bu-Ali Sina University, Hamedan, Iran

Background: Cutaneous leishmaniasis is one of the common diseases with a parasitic origin and relatively high prevalence in endemic areas. So far, no effective prevention methods have been introduced for this disease. The aim of this study was to investigate the immunogenicity caused by macrophages exposed to Leishmania passage one parasite in-vivo, the results of this study can be used to design a vaccine based on the virulence reduction strain.

Methods: Passage of a standard strain of *Leishmania major* parasite was injected into BALB\c female mice aged 6-8 weeks, after wounding on days (1, 15, 30, 45), phagocytosis of mouse peritoneal cavity macrophages infected with passage one parasite, were examined by NBT (Nitro Blue Tetrazolium Test) and the expression pattern of pro-inflammatory and inflammatory genes TGF-β IFN-γ, IL- 10, IL-17, IL-4, IL-1α AIRE, TNF-α IL-12P40, IL-12p35 were evaluated before and after ingestion.

Results: The lesion of infected mice did not heal until the end of the period and phagocytosis rate was different in mice infected with passage one parasite, this difference was not significant $p<0.05$. IL-10, AIRE, TGF-β and TNF-αgenes were expressed in macrophages exposed to passage one parasite.

Conclusion: Considering the lack of change in the amount of phagocytosis during the infection, the increase in the expression pattern of immune response modulating genes in infected mice, the deterioration of the skin wounds was clearly evident could be due to the insufficiency of the immune responses via more activity of Th2 in passage 1 of the parasite infection.

Keywords: *Leishmania major*, macrophage, phagocytosis, inflammatory genes

Investigation of IL-6 in Patients with Ocular Toxoplasmosis

Seyed Parsa Dastvarz[1], Mohammad Zarei[2], Shima Hosseinzadeh[1], Bahman Rahimi Esboei[3]

1. Student research Committee, School of Medicine, Tonekabon branch, Islamic Azad University, Tonekabon, Iran.
2. Retina Service, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Medical Parasitology, School of Medicine, Tonekabon branch, Islamic Azad University, Tonekabon, Iran.

Background: Ocular toxoplasmosis (OT) is a parasitic infection in the eye that causes uveitis and retinocorticoiditis in humans, which is caused by the parasite *Toxoplasmo gondii*. The severity of OT infection varies in different patients and this is probably due to the state of the immune system. Various cytokines were examined in these patients, but the cytokine profile in these patients has not well defined yet. In this study, the level of IL-6 in patients with OT compared to the control group was investigated using the Real Time PCR method.

Methods: In this experimental study, 34 patients with OT infection and 20 patients with cataract were included in the study as a control group. The vitreous sample was taken from the patients in the operating room. After extracting RNA, the real time PCR reaction was performed and the expression level of IL-6 gene was investigated.

Results: The results of this study showed that among 34 patients with OT, the level of IL-6 gene expression increased in 3 patients and decreased in 22 patients. In 20 patients with cataract, the level of expression was increased in 1 patient, decreased in 3 patients, and normal in 16 patients. The results of the gene expression level showed that the expressed level of IL-6 in patients with OT was significantly higher than it has decreased to the patients of the control group (cataract).

Conclusion: The results have shown that IL-6 has decreased in patients with OT and it is possible to control the severity of the disease in patients with OT by conducting additional studies.

Keywords: Ocular Toxoplasmosis, *Toxoplasma gondii*, IL-6, Real time PCR

Polymeric encapsulated antigens from *Echinococcus granulosus* as a potential anti tumor agent: A systematic review

Soheil Sadr[1], Hadi Mohebalian[2], Abbas Rahdar[3], Nooshinmehr Soleymani[2], Hassan Borji[2]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Physics, University of Zabol, Zabol

Background: *Echinococcus granulosus* is a parasitic tapeworm that causes echinococcosis or hydatid disease in humans and livestock. The larval stage of the parasite forms hydatid cysts in the liver, lungs, and other organs, which can cause serious health complications. This review aims to investigate previous literature related to tumors and *Echinococcus granulosus*.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on *Echinococcus granulosus* anti-tumor effects from January 2000 to January 2023. Fifteen affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: The anti-tumor effects of polymeric encapsulated antigens have been demonstrated in various types of cancer, including colon, lung, and breast cancer. One of the mechanisms by which polymeric-encapsulated antigens exert their anti-tumor effects is inducing apoptosis or programmed cell death in cancer cells. This leads to the death of cancer cells, which helps to slow or stop the growth of tumors.In addition to inducing apoptosis, polymeric-encapsulated antigens have also been shown to inhibit cell proliferation in cancer cells. The results showed that the encapsulated antigens could induce an immune response in mice and significantly reduce tumor growth. These findings suggest that polymeric encapsulated antigens from *Echinococcus granulosus*, including AgB and Ag5, have potential as an anti-tumor agent and warrant further investigation.

Conclusion: Although the anti-tumor effects of polymeric encapsulated antigens are promising, further research is needed to understand its potential as a cancer treatment fully and to optimize its use in clinical settings. Overall, the findings suggest that polymeric encapsulated antigens may be a promising candidate for cancer treatment and warrant further investigation.

Keywords: Antitumor, Cancer immunotherapy, *Echinococcus granulosus*, Nanotechnology

Prevalence of Asymptomatic Strongyloidiasis Co-Infection in COVID-19 Patients Residing in Endemic Areas

Alireza Ashiri[1], Molouk Beiromvand[1], Abdollah i Rafie[1]

1. Department of Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: Fatal forms of strongyloidiasis, hyperinfection syndrome (HS) and disseminated strongyloidiasis (DS), are caused by an exaggerated autoinfection of the intestinal nematode, *Strongyloides stercoralis (S. stercoralis)*. The use of corticosteroids, common in patients with severe COVID-19, can transform chronic asymptomatic strongyloidiasis into the aforementioned fatal diseases. This study aimed to investigate the prevalence of strongyloidiasis in COVID-19 patients receiving corticosteroids in a hypoendemic region.

Methods: The present cross-sectional study enrolled 308 COVID-19 patients admitted to two hospitals in Ahvaz and Abadan in the southwest of Iran between 2020 and 2022. A real-time reverse transcription polymerase chain reaction (RT-PCR) test and chest computed tomography (CT) scan were employed to detect and monitor the disease's severity, respectively. All patients were evaluated for IgG/IgM against *S. stercoralis* using Enzyme-Linked Immunosorbent Assay (ELISA). Afterward, individuals with a positive ELISA test were confirmed using parasitological methods, including direct smear and agar plate culture (APC).

Results: The age range of the participants was 15–94 years, with a mean age of 57.99±17.4 years. Of the 308 patients, 12 (3.9%) had a positive ELISA test, 4 (1.3%) were equivocal, and 292 (94.8%) had negative result. Three of the 12 patients with a positive ELISA result died, and three did not provide a stool sample. To this end, only six cases were examined parasitologically, and *S. stercoralis* larvae were detected in five patients. Furthermore, significant correlations were observed between *S. stercoralis* infection with gender (p=0.043) and age (p=0.039).

Conclusion: Our findings indicated that screening for latent strongyloidiasis using diagnostic methods with high sensitivity, particularly ELISA, in COVID-19 patients in endemic regions before receiving suppressive drugs should be given greater consideration.

Keywords: Strongyloidiasis; COVID-19; Eosinophilia; ELISA; Khuzestan Province; Iran

Seroepidemiological investigation on coinfection of toxoplasmosis and tuberculosis in Northern Iran: A case control study

Reza Jafari-Shakib[1], Abbas Sadeghi[2], Bijan Majidi-Shad[2], Zahra Atrkar-Roshan[3], Meysam Sharifdini[2]

1. Department of Immunology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
2. Department of Medical Parasitology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
3. Department of Biostatistics, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

Background: Tuberculosis (TB) and parasitic coinfections in human is prevalent in developing countries. We investigate the seroprevalence of *Toxoplasma gondii (T. gondii)* infection among TB patients in Guilan province.

Methods: In a case–control study, anti-toxoplasma antibodies was measured in 100 active TB patients by ELISA method and compared with 100 healthy controls who matched by age, sex, and place of residence.

Results: Anti-*T. gondii* IgG antibodies were detected in 62% of active TB patients and 70% of control individuals. The seroprevalence was not significant statistically between two groups (p>0.05). Anti-*T. Gondii* IgM antibodies was diagnosed in 1% of both groups. Sociodemographic and behavioral factors was not recognized as risk factor of toxoplasmosis in TB infected patients. Moreover, level of anti-*T. gondii* IgG antibodies titer in TB patients was significantly higher than control individuals.

Conclusion: This study showed prevalent coinfection of toxoplasmosis and tuberculosis in Guilan province and due to similar clinical presentation in sometimes between these two diseases, it should be considered in the clinical situations. One reason for the lack of seroprevalence difference between two groups in our study in comparison to other studies can be high prevalence of toxoplasmosis in our study area. The underlining immune mechanism caused higher titer of anti-*T. gondii* IgG in TB patients needs to be explored but it may indicate deviation to humoral response in active tuberculosis.

Keywords: Toxoplasmosis, Tuberculosis, Coinfection

Seroprevalence and associated risk factors of toxoplamosis among pregnant women in Ramsar city

Reza Jafari-Shakib[1], Abbas Sadeghi[2], Bijan Majidi-Shad[2], Zahra Atrkar-Roshan[3], Meysam Sharifdini[2]

1. Department of Immunology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
2. Department of Medical Parasitology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
3. Department of Biostatistics, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

Background: Toxoplasmosis is a zoonotic parasitic infection with high prevalence and worldwide distribution in human beings. It can cause severe disease in fetus of newly infected pregnant women and immunodeficient patients. This study was aimed to evaluate the seroprevalence of toxoplasmosis and its related risk factors among pregnant women in Ramsar city.

Methods: A cross-sectional descriptive study was conducted. Samples of peripheral blood from pregnant women were collected for the detection of specific anti- *Toxoplasma gondii* IgG, IgM and IgG avidity through ELISA. The prevalence of toxoplasmosis was measured among 191 pregnant women attended to the pregnancy care clinic in Ramsar during the period from October 2017 to March 2018. Demographic and behavioral information of pregnant women were collected through interviews.

Results: The overall prevalence of *T.gondii* IgG, IgM and both IgG & IgM seropositivity was 46%, 5.8% and 4.7%, respectively. IgG avidity test didn't show any acute toxoplasmosis in our participants. Demographic characteristics, such as age, occupation, place of residence, education, income, trimesters of pregnancy, frequency of pregnancy and history of abortion and behavioral characteristics such as source of consumed water, contact with soil, presence of cats in household, consumption of vegetables, type of meat consumed and the way of meat cooking had no significant correlation with toxoplasmosis seropositivity.

Conclusion: More than half of the pregnant women were at risk of acquiring toxoplasmosis. Educating the pregnant women about toxoplasmosis and ways to prevent it can help reduce the risk of infection during pregnancy.

Keywords: *Toxoplasma gondii*; seroprevalence; risk factors; pregnancy

Seroprevalence of specific antibodies against *Toxoplasma gondii* among blood donors in Abadan and Khorramshahr cities in 2021

Shahrzad Soltani[1], Mehdi Sagha-Kahvaz[1], Haniyeh Badpa[1], Masoud Foroutan[1], Fatemeh Maghsoudi[1], Mohammad Amin Shaverdi[1]

1. Abadan University of Medical Sciences, Abadan, Iran.

Background: *Toxoplasma gondii* is one of the most common parasites between humans and other vertebrates worldwide. Most cases of toxoplasmosis are asymptomatic and rarely show symptoms which can make diagnosis and transmission control difficult. Serological screening allows early detection and adverse consequences in blood recipients can be avoided. The aim of this study is to investigate the prevalence of acute and chronic toxoplasmosis in blood donors in Abadan and Khorramshahr during the year 2021.

Methods: The current cross-sectional descriptive study was conducted on the population of 345 healthy people who referred to Abadan and Khorramshahr Blood Transfusion Organization in 2021 to donate blood. ELISA test was performed to check the presence of IgG and IgM antibodies against Toxoplasma. The results of the questionnaires were analyzed using SPSS version 22 software and Chi-square statistical test.

Results: In this study, the prevalence of Toxoplasma infection was 31.88% among the blood donor volunteers. No statistically significant correlation was observed between consumption of raw/semi-cooked meat, unwashed vegetables, contact with cats, contact with soil, O+ blood type and positive IgG (P>0.05).

Conclusion: As a result, the prevalence of *T. gondii* antibody in blood donors in Khorramshahr and Abadan was estimated at 31.88%, which was higher than other countries. There is still a risk of contracting toxoplasmosis through blood transfusions. Seroprevalence does not mean active Toxoplasma infection, but since most blood recipients are high-risk patients, it is necessary to screen donated blood for *T. gondii* in Iran's blood transfusion organization, including Khoramshahr and Abadan.

Keywords: *Toxoplasma gondii*, prevalence, ELISA, risk factors, Khorramshahr, Abadan

Seroprevalence, potential risk factors and clinical symptoms of *Toxocara canis* infection among general population: a community-based study in southwest Iran

Masoud Foroutan[1], Shahrzad Soltani[1], Naser Kamyari[1], Ehsan Moradi-Joo[1], Milad Badri[2], Aida Vafae Eslahi[2]

1. AbaAbadan University of Medical Sciences, Abadan, Irandan University of Medical Sciences
2. Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

Background: Human toxocariasis (HT) is a helminthic zoonotic infection with a worldwide distribution, particularly in countries and territories with poor standard of hygiene and sanitation. Despite health promotion actions during recent decades, the risk of acquiring HT remains a challenge for public health in developed and developing countries. This study was designed to estimate the seroprevalence, risk factors, and clinical symptoms of *Toxocara canis (T. canis)* infection in the general population.

Methods: In the present cross-sectional investigation, a total of 360 individuals were enrolled to participate from both rural and urban communities of southwest Iran (Khorramshahr and Abadan counties, Khuzestan Province) in 2022. In the next step, all serum samples were screened to detect anti-*T. canis* IgG antibodies through ELISA. Also, we used a structured questionnaire to gather demographic variables, potential risk factors, and related clinical manifestations of *T. canis* infection.

Results: Following serological assay, 12.77% (46/360) of participants were found to be seropositive. Data analysis was revealed that some risk factors including, residing in rural regions, ingestion of unwashed vegetables, drinking unpurified water, contact with dog and soil were significantly associated with HT. Based on clinical symptoms, *T. canis* infection was significantly associated with ophthalmic disorders or blurred vision (odds ratio [OR] = 16.67, 95% CI = 9.09 – 33.33, $p<0.001$), skin allergic disorders (OR = 6.25, 95% CI = 3.33 – 12.50, $p< 0.001$), and asthma (OR = 5.26, 95% CI = 2.86 – 10.0, $p<0.001$).

Conclusion: The present report provide useful baseline sero-epidemiological results regarding HT among general population of southwest Iran that can be employed by policy makers to control the infection among different groups of population, especially high risk subjects.

Keywords: *Toxocara canis*, Seroprevalence, General population, Risk factors, Iran

SLA-R848-Pam3CSK4 combination as a therapeutic vaccine for treatment of experimental cutaneous leishmaniasis

Asal Katebi[1], Farhad Riazi-rad[1], Soheila Ajdary[1]

1. Department of Immunology, Pasteur Institute of Iran, Tehran, IR-Iran

Background: Leishmaniasis is a vector-borne disease affecting 12 million people in the world. Because of the high prevalence of this disease and also problems related to the control and therapy of leishmaniasis, the development of effective and applicable therapeutic approaches in the treatment of leishmaniasis seems to be essential. In recent years, therapeutic vaccines have been considered promising approaches against leishmaniasis. So, we examined the therapeutic efficacy of Soluble Leishmania antigen (SLA) in combination with TLR agonists (R848 and Pam3CSK4) as adjuvants using the BALB/c mice model.

Methods: To develop a new therapeutic vaccine for leishmaniasis, SLA and/or Pam3CSK4 and/or R848 were injected one week after infection three times. One week after the infection, footpad swelling was monitored weekly. Parasite burden was also assessed by serial dilution 11 weeks post-infection. Before and after vaccination, blood samples were collected, and humoral responses were evaluated using an ELISA assay. Cytokines and NO production were analyzed 11 weeks post-infection in all groups.

Results: Immunological analysis showed that mice treated with SLA-R848-Pam3CSK4, were able to control cutaneous leishmaniasis disease and subsequently the smallest lesion size, decreased parasite load, increased IgG2a, IgG2a/IgG1, IFN-γ, and NO production.

Conclusion: The results revealed the effectiveness of SLA-R848-Pam3CSK4 modulation as a therapeutic vaccine in infected BALB/c mice against *Leishmania major* infection.

Keywords: R848, Pam3CSK, *Leishmania major*, Therapeutic vaccine

The assessment of C57BL/6 mice-derived exosome effect on polarization and parasite killing activity of infected BALB/c macrophages with *Leishmania major*

Mohammad Mahdi Jafari[1], Ahmad Zavaran Hosseini[1]

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background: Leishmaniasis is a major global health organization problem. Current treatments for leishmaniasis have many problems, so better treatments are needed. Macrophages play an effective role in dealing with parasites. exosomes that secreted from various cells, such as macrophages, may exert M1(classically activated) or M2 (alternative activated) phenotype in other macrophages, so in this study, the effect of C57BL/6 mouse-derived exosomes on polarization and parasite killing activity of infected BALB/c macrophages with *Leishmania major* was investigated.

Methods: The *Leishmania major* parasite was cultured in an enriched Roswell Park Memorial Institute (RPMI) 1640 culture medium (10% fetal bovine serum (FBS)). Exosomes were separated from the peritoneal supernatant using the Exocib kit. The size and shape of exosomes were determined using DLS (Dynamic light scattering) and a FESEM (Field emission scanning electron microscopy). Peritoneal macrophages cultured in an enriched RPMI medium. Parasites and exosomes were added to cultured macrophages. All groups were stained with Giemsa dye, and the cell soup of the macrophages was used to check the amount of nitric oxide using a nitrocib kit and inflammatory/anti-inflammatory cytokines using an Enzyme-linked immunosorbent assay (ELISA).

Results: The results of the DLS test and FESEM microscope approved the spherical shape and 80-100 nm size of the exosomes. The results of staining macrophages showed that the Macrophage BALB/c +Exosome C57BL/6+ *Leishmania major* group has low number of infected macrophages and low parasite number in each macrophage. Also, this group has high amounts of nitric oxide and inflammatory cytokines (TNF α(Tumor necrosis factor alpha), IL-6 (interleukin 6) and low amounts of anti-inflammatory cytokines (TGF β (Transforming growth factor beta), IL -10 (Interleukin 10)).

Conclusion: The C57BL/6 mice-derived exosomes have a stimulatory effect on BALB/C macrophages, and drive the macrophages towards the M1 phenotype, which is associated with increasing parasite killing activity.

Keywords: Macrophage, *Leishmania major*, exosome, Balb/c mice, C57BL/6 mice

The immune response and survival rate against *Toxoplasma gondii* in BALB/c mice induced by nanoliposome containing soluble antigens

Ahmad Mehravaran[1]

1. Department of Parasitology, School of Medicine, Zahedan University of Medical Science, Iran

Background: Toxoplasmosis is one of the most common parasitic infections worldwide induced by *Toxoplasma gondii* (*T. gondii*). This study aimed to compare the immune response and survival rate against *Toxoplasma gondii* in BALB/c mice induced by nanoliposome containing soluble antigens (SA).

Methods: In the present research, a nano-liposomal vaccine containing soluble antigens (SA) was designed to evaluate the immunity and protective efficacy against *T. gondii* infection in BALB/c mice. Soluble antigens (SA) were achieved from tachyzoites, encapsulated in the liposome, and investigated via scanning electron microscope. Three times with 2-week intervals, BALB/c mice were immunized subcutaneously with different formulations. The level of protection against infection was assessed through the percent survival survey of BALB/c mice after challenge with tachyzoites of *T. gondii* RH strain; also, the type of generated immune response was determined by evaluating the generation of cytokine (IFN-γ, IL-4) and titration of IgG isotypes.

Results: The immunization with liposome DSPC+ SA and liposome DSPC+ Imiquimod + SA induced a substantial increase in anti-Toxoplasma IgG antibody as compared to the PBS group (p <0.05). The IgG2a and IFN-γsecretion highest levels were seen with liposome DSPC+ Imiquimod + SA more than the control group (p<0.01) and (p<0.0001), respectively. After challenge with tachyzoites, less mortality was detected in the immunized mice by liposome DSPC + Imiquimod + SA that was meaningfully different (p <0.01) in comparison to other groups.

Conclusion: This project evidenced that liposome DSPC + Imiquimod + SA showed more survival rate and cellular immune reaction against *T. gondii*.

Keywords: Immune response. Survival rate. *Toxoplasma gondii*. Nanoliposome.

The Master of Cancer Negative Regulator, *Trichinella Spiralis*: a systematic review

Macan Shafiei[1], Soheil Sadr[1], Pouria Ahmadi Simab[2], Hassan Borji[3]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
3. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: In recent years, there has been noticeable interest in utilizing biological, viral, bacterial, yeast, and parasitic agents to cure cancers. According to several studies, parasitic infections and the occurrence of neoplasms are negatively correlated, as well as their interference with the growth of tumors. This review aims to investigate previous literature related to tumors and *Trichinella spiralis*.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on *T. spiralis* anti-tumor effects from January 2000 to January 2023. Fifteen affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: *T. spiralis* was found to have antitumor properties. The active proteins in *T. spiralis*, such as Caveolin-1, Heat shock proteins, and Ribosomal proteins, are thought to inhibit cancers such as melanoma, myeloma, sarcoma, leukemia, stomach cancer, colon cancer, breast cancer, and lung cancer. In addition, these proteins are thought to induce apoptosis in specific neoplastic cells. Excretory-secretory proteins (ESPs) are complex proteins produced by *T. spiralis* during the infestation. It is believed that polypeptide proteins and ESPs may inhibit tumor growth during the life cycle of *Trichinella spiralis*, including the muscle larva (ML), the newborn larva (NBL), and the adult worm.

Conclusion: Our knowledge of *T. spirals'* role in antitumor therapy has greatly improved due to advancements in research on the relationships between the organism and tumors. *Trichinella spiralis* may be an effective tumor biotherapy agent, but many unanswered questions remain.

Keywords: Antitumor, Apoptosis, Cancer immunotherapy, *Trichinella spiralis*

Echinococcus granulosus cell-free DNA in the serum of hydatid cysts patients as a diagnostic indicator

Fatemeh Faghihiyan Alirezaei[1]

Background: Hydatid cyst is a zoonotic disease common between humans and livestock worldwide. The larvae stage of *Echinococcus granulosus*, a cestode helminth, causes this disease. In new research, efforts are being made to find the parasite's cell-free DNA in the patient's body fluids using molecular methods. This study aimed to propose a method to diagnose the disease based on finding the cell-free DNA ofthe parasite using the mitochondrial gene rrnS in the serum of patients.

Methods: The serum of 101 patients with hydatid cysts was included in the study. The DNA of serum samples was extracted using the by QIAamp DNA Blood Minikit.The *E. granulosus* DNA detection was performed by conventional and real-time PCR by amplifying the rrnS gene.

Results: DNA of *E. granulosus* was detected in the sera of 32.7% (95% CI: 24.3–42.3) and 57.6% (95% CI: 47.7–66.8) of 101 and 99 patients by conventional and real-time PCR, respectively. The specificity and sensitivity of the rrnS gene are 100% (95% CI: 69.2–100) and 32.7% (95% CI: 23.7–42.7) for conventional PCR and 100% (95% CI: 83.2–100) and 57.6% (95% CI: 47.2–67.5) for real-time PCR respectively.

Conclusion: Our finding reveals that the rrnS gene of *E. granulosus* for detecting cell-free DNA has good sensitivity and specificity for diagnosing Hydatid cysts. Therefore, the cell-free DNA of *E. granulosus* as a noninvasive diagnostic method can be considered a diagnostic indicator in the serum of Hydatid cyst patients with real-time PCR. However, this procedure needs more modification to be applicable in the medical laboratory.

Keywords: cfdna - cyst hydatic

Trained immunity in Leishmania infection following BCG administration

Mahmoud Nateghi Rostami[1], Yahya Sohrabi[2], Negin ElahiRad[1], Zohreh Askaripour[1]

1. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran
2. Department of Cardiology – Coronary and Peripheral Vascular Disease, Heart Failure, University Hospital Munster, Munster, Germany

Background: Trained immunity induces enhanced inflammatory and antimicrobial properties in innate immune cells, conferring nonspecific protection to unrelated infections. Here, we investigate whether BCG-induced trained immunity is able to protect against infections caused by Leishmania parasites.

Methods: BALB/c mice were injected intraperitoneally (i.p.) or intravenously (i.v.) with 40 µg/mouse of Bacillus Calmette–Guerin (BCG, ~2 × 105 CFU/mL). Peritoneal macrophages and bone marrow (BM) immune cells were isolated and resuspended in RPMI 1640 medium and restimulated with 15 µg/mL of lipopolysaccharide (LPS), 5 µg of soluble *L. major* or *L. tropica* antigen at 5% CO2 incubator. Cells were collected after 6 hrs of incubation for mRNA levels of IL-1β TNF and IL-6 as well as metabolic enzymes of glycolytic pathway including hif-1, pfkb-3 and hk-3 using real time RT-PCR assay. Supernatants were harvested after 24 hrs of incubation and TNF and IL-6 production was determined using sandwich ELISA. After 2 weeks of i.v. injection, mice were infected with 1 × 106 L. major promastigotes subcutaneously. Lesion size was measured weekly and spleen/lymph nodes were isolated for parasite burden assay. MQ infectivity assay was done by infection of trained and non-trained macrophages with stationary-phase promastigotes of either *L. major* or *L. tropica*.

Results: Monocytes trained with BCG presented enhanced ability to kill *L. major* and *L. tropica*. BCG exposure induced IL-1βand TNF production in mice monocytes, at both mRNA and protein levels. Also, BCG vaccination decreased lesion size and reduced the parasite load tissues of mice infected with L. major. We observed significantly increased expression of hif-1a, pfkp3, and hk3 mRNA expression in BM cells from BCG injected mice.

Conclusion: BCG's ability to train innate immune cells might benefit the development of effective vaccine against human leishmaniasis. Interleukin IL-1β and TNF appears to contribute in the development of trained immunity.

Keywords: Leishmaniasis, trained immunity, TNF, IL-1β BCG

Immunovirology

Association between human herpesviruses and multiple sclerosis: A systematic review and meta-analysis

Arash Letafati[1]

1. Tehran University of Medical Sciences, Tehran, Iran.

Background: The aim of this study was to investigate the prevalence and potential association between infection with different herpes viruses and multiple sclerosis (MS).

Methods: A systematic literature search was performed by finding relevant cross-sectional and case-control studies from a large online database. Heterogeneity, Odds ratio (OR), and corresponding 95% Confidence interval (CI) were applied to all studies by meta-analysis and forest plots. The analysis was performed using Stata Software v.14.

Results: One hundred and thirty-four articles (289 datasets) were included in the meta-analysis, 128 (245 datasets) of which were case/control and the rest were cross-sectional. The pooled prevalence of all human herpes viruses among MS patients was 50% (95% CI: 45–55%; I2 = 96.91%). In subgroup analysis, the pooled prevalence of Herpes simplex virus (HSV), Varicella-zoster virus (VZV), Epstein–Barr virus (EBV), Cytomegalovirus (CMV), Human herpes virus 6 (HHV-6), Human herpes virus 7 (HHV-7), and Human herpes virus 8 (HHV-8) was 32%, 52%, 74%, 41%, 39% 28%, and 28%, respectively. An association was found between infection with human herpes viruses and MS [summary OR 2.07 (95% CI (1.80–2.37); I2 = 80%)].

Conclusion: The results of the present study showed that EBV, VZV, and HHV-6 infection are associated with multiple sclerosis and can be considered as potential risk factors for MS. Although the exact molecular mechanism of the role of herpes viruses in the development of MS is still unknown, it seems that molecular mimicry, the release of autoreactive antibodies, and inflammation in the CNS following viral infection can be important factors in the induction of MS.

Keywords: MS, Immunology, Virology, Herpes viridae

Association between human polyomavirus infection and brain cancer: A systematic review and meta-analysis

Arash Letafati[1]

1. Tehran University of Medical Sciences, Tehran, Iran.

Background: The aim of this study was to investigate the prevalence and potential association between the infection with some members of the polyomaviridae family of viruses and development of the brain tumors.

Methods :A systematic literature search was performed by finding relevant cross-sectional and case-control studies from a large online database. Heterogeneity, OR, and corresponding 95% CI were applied to all studies by meta-analysis and forest plots. The analysis was performed using Stata Software v.14.

Results :Twenty-three articles (33 datasets) were included in the meta-analysis, four (four datasets) of which were case/control studies and the rest were cross-sectional. The pooled prevalence of polyomaviruses among brain cancer patients was 13% (95% CI: 8–20%; I2 = 96.91%). In subgroup analysis, the pooled prevalence of JCV, SV40, BKV and Merkel cell polyomavirus was 20%, 8%, 6%, and 16%, respectively. An association was found between polyomavirus infection and brain cancer [summary OR 7.22 (95% CI (2.36–22.05); I2 = 0%)]. The subgroup analysis, based on the virus type, demonstrated a strong association between JCV infection and brain cancer development [summary OR 10.34 (95% CI 1.10–97.42; I2 = 0%)].

Conclusion :The present study showed a significant association between polyomavirus infection and brain tumors. Moreover, these results suggest that polyomavirus infection may be a potential risk factor for the development of brain cancer.

Keywords: Virology, Immunology, Brain Cancer, Polyomavirus

Association between Rotavirus Infection and Irritable Bowel Syndrome: A Case-control Study in Iran

Mostafa Aalipour[1], Mohammad Javad Zahedi[2], Mohammad Reza Zangouey[1], Abolfazl Yari[3], Moghaddameh Mirzaee[4], Mohammad Mahdi Mohammadi[1]

1. Department of immunology, Afzalipour Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
2. Department of Internal medicine, Kerman University of Medical Sciences, Kerman, Iran.
3. Cellular and Molecular research center. Birjand University of Medical Sciences, Birjand, Iran.
4. Department of Biostatistics and Epidemiology, School of Public Health, Kerman University of Medical Sciences, Kerman, Iran.

Background: Irritable bowel syndrome is a functional gastrointestinal disease of unknown etiology. Researchers have recently drawn attention to the possible role of viruses in the development of IBS and provided evidence in this regard. In this study, it was decided to investigate the possible role of rotavirus infection in the onset of IBS.

Methods: Stool and serum samples were collected from 40 patients with IBS and 40 healthy individuals. To evaluate the presence and concentration of anti-rotavirus IgG, ELISA test was performed on the serum samples. Real-time PCR test was also used to measure the viral load in the stool. Finally, the data were analyzed by SPSS-22 software.

Results: No significant relationship was found between anti-rotavirus IgG in the serum of case and healthy individuals. Moreover, there was no significant difference between the viral genome load in the stool samples of the two groups.

Conclusion: According to the results, it seems unlikely that a link exists between rotavirus infection and the onset of irritable bowel syndrome, but the possible role of other gastrointestinal viruses, including coronavirus, remains.

Keywords: Irritable Bowel Syndrome, Rotavirus, Pattern Recognition Receptors, Coronavirus.

Cross-reactivity of HBe antigen specific polyclonal antibody with HBc antigen

Maryam Hojatizadeh[1], Forough Golsaz-Shirazi[1], Fazel Shokri[1], Mohammad Mehdi Amiri[1], Mahmood Jeddi-Tehrani[2]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACER, Tehran, Iran

Background: Hepatitis B virus (HBV) infection is a major health problem worldwide and causes almost one million deaths annually. HBV core gene codes for two related antigens, known as core antigen (HBcAg) and e-antigen (HBeAg), sharing 149 residues but having different amino- and carboxy-terminals. HBeAg is a soluble variant of HBcAg and a clinical marker for determining the disease severity and patients screening. Currently available HBeAg assays have a shortcoming of showing cross-reactivity with HBcAg. Here, for the first time, we evaluated whether HBcAg-adsorbed anti-HBe polyclonal antibodies could specifically recognize HBeAg or still show cross-reactivity with HBcAg.

Methods: Recombinant HBeAg was cloned in pCold1 vector and successfully expressed in Escherichia coli (E. coli) and after purification by Ni-NTA resin was used to generate polyclonal anti-HBe antibodies in rabbit. Purified HBeAg was further characterized by assessing its reactivity with anti-HBe in the sera of chronically infected patients and HBeAg-immunized rabbit.

Results: Sera from patients with chronic HBV infection, containing anti-HBe, specifically reacted with recombinant HBeAg, implying that the conformation of prokaryotic recombinant HBeAg was conserved and it contained similar immunogenic epitopes to the native HBeAg in the serum of HBV infected patients. In addition, the designed enzyme-linked immunosorbent assay (ELISA) with rabbit anti-HBe polyclonal antibodies could detect recombinant HBeAg with high sensitivity, while high cross-reactivity with HBcAg was observed.

Conclusion: It is noteworthy that HBcAg-adsorbed anti-HBe polyclonal antibodies still showed high cross-reactivity with HBcAg, implying that due to the presence of highly similar epitopes in both antigens, HBcAg-adsorbed polyclonal antibodies cannot differentiate between the two antigens.

Keywords: Hepatitis B e antigen (HBeAg), Hepatitis B virus (HBV), Anti-HBe polyclonal antibody

Epstein-Barr virus infection and toll-like receptor 9 gene variants affect the susceptibility of Iranian individuals to rheumatoid arthritis

Kurosh Kalantar[1], Fatemeh Nezhadshamohammadi[1], Sara Mirzazadeh[1], Gholamreza Daryabor[1]

1. Shiraz University of Medical Sciences, Shiraz, Iran.

Background: Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease. Although the exact cause of RA is obscure, genetics and environmental factors play a crucial role in developing the disease. This study pointed to explore the conceivable links between toll-like receptor 9 (TLR9) single nucleotide polymorphisms (SNPs) and the risk of RA or Epstein-Barr infection (EBV) disease, besides the plausible interface between EBV infection, TLR9 gene variants, and susceptibility to RA among Iranian population.

Method: TLR9 SNPs rs187084 and rs352140 were assessed by polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) for 213 RA patients and 235 healthy individuals. Furthermore, EBV DNA was detected by real-time PCR.

Results: The frequency of rs352140 CT and TT+CT genotypes was significantly higher in healthy individuals compared with RA patients (p=0.01 and p=0.02, respectively), while the TC/TC haplotype combination was increased in RA patients (p= 0.01). Also, a higher frequency of EBV infection was determined in RA patients than in the controls (p=0.04). In addition, EBVneg infected healthy subjects revealed a significantly elevated frequency of the rs187084 C allele, especially in females (p=0.05 and p=0.04, respectively).

Conclusion: According to our results, the combination of rs352140 CT genotype and rs352140 CT+TT genotype plays a protective role against RA, whereas EBV infection and TC/TC haplotype combination might predispose to susceptibility to RA. Moreover, developing RA might be prevented in EBVneg infected individuals through the rs187084 C allele.

Keywords: Keywords: Autoimmunity, Epstein-Barr virus, Rheumatoid arthritis, single nucleotide polymorphisms, toll-like receptor 9.

Frequency of Anti-Rubella IgG and IgM in Rubella Suspected Patients in Sari, during 2022-2023

Amirhossein Rafinezhad[1], Mahdi Shooraj[1], Mohammadreza Nadernezhad[2], Yahya Ehteshaminia[3], Seyedeh Farzaneh Jalali[4], Mohammad Reza Mahdavi Amiri[5], Seyed Ehsan Seyed Ehsan[6], Hossein Mokhtari[7], Maysam Rezapour[8], Hadi Hassammia[9]

1. Student Research Committee, Amol School of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Hematology and Medical Laboratory Sciences, Faculty of Allied Medicine, Kerman University of Medical Sciences, Kerman, Iran
5. Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran
6. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
7. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. Department of Paramedicine, Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran.
8. Department of Paramedicine, Amol Faculty of Paramedical Sciences, Mazandaran University of Medical Sciences, Sari, Iran
9. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

Background: Rubella is a virus that generally causes a temporary illness, but infection during pregnancy, particularly during the first trimester can lead to miscarriage, fetal death, stillbirth, or infants with a rare disorder known as Congenital Rubella Syndrome (CRS). The aim of this study was to investigate the immunity against Rubella by evaluating anti-rubella IgG and IgM levels in examined population.

Methods: In a cross-sectional study 2565 blood samples were collected from patients and concentration of serum anti-rubella IgG and IgM were determined by Enzyme-Linked Immunosorbent Assay (ELISA). Participants were categorized based on their age group, gender and anti-rubella IgG and IgM titer. Formerly, the results were analyzed via GraphPad Prism (v9.0.0) software.

Results: A total of 2565 samples, including 39 males (1.5%) and 2526 females (98.5%) from 5 age groups were included in this study. Based on the data analysis, frequency of anti-rubella IgG and IgM positive samples in examined population was 90.8% and 0.6% respectively. Accordingly, 81% (N=17) of males and 90.1% (N=1267) of females were anti-rubella IgG positive. Otherwise, all of the patients with positive anti-rubella IgM were females at the 20-39 age group.

Conclusion: This study revealed that 1/10 of females and 2/10 of men were anti-Rubella IgG negative in examined population. Moreover, the frequency of anti-Rubella IgM positive samples was observed 1/225. Anti-Rubella IgM results represent the exposure probability and highlight the associated risk for pregnant women. Hence, assessment of anti-Rubella immunoglobulins as a screening program, especially for women in pregnancy age is remarkable.

Keywords: Congenital Rubella Syndrome, Anti-Rubella immunoglobulins, Pregnancy

Higher expression of Cannabinoid receptor 2 (CB2R) in patients with HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP)

Afsaneh Fadaee[1], Fatemeh Sadat mohammadi[1], Nazila Ariaee[1], Seyed Abdolrahim Rezaee[4]

1. Immunology Research Center, Inflammation and Inflammatory Diseases Division, Mashhad University of Medical Sciences, Mashhad, Iran

Background: HTLV-1 is the causative agent of a neurodegenerative disease, (HAM/TSP). As the role of endocannabinoid system confirmed in neuro-inflammatory diseases such as multiple sclerosis, in this study two types of cannabinoid receptors, CB1R and CB2R and their associations with HTLV-1 proviral load (PVL) were evaluated.

Methods: A cross-sectional study was performed on 19 people with HAM/TSP, 22 asymptomatic carriers (ACs) and 18 healthy controls (HCs). The expression level of CB1R and CB2R genes was measured using RT-qPCR technique. Proviral load was also measured in two groups of HTLV-1 infected subjects.

Results: The results showed that the expression of CB1 and CB2 receptors in ACs was increased compared to HAM/TSP patients and HCs subjects. Regarding the CB1R gene in the HCs group, it is significantly lower than both ACs groups (p=0.001) and HAM/TSP patients (p=0.01), but increased level in ACs which was nor significant. Furthermore, the results of CB2R gene expression showed that the level of CB2R expression in ACs is strongly higher compared to HAM/TSP patients (p=0.001) and HCs (p=0.006). The results of comparison of PVL between HAM/TSP patients and ACs is statistically significant (p=0.045). There were significant correlations between HCs and HAM/TSP However, there was no correlation between PVL with CB1 or CB2 in studied groups. Since the distribution of endogenous cannabinoid receptors in the body is selective, i.e. CB1R is more expressed on neurons with neuroprotective effects and CB2R is selectively, on immune cells with the effect of immuno-modulatory properties.

Conclusion: The present study, showed that in neurological disease related to HTLV-1 infection (HAM/TSP) with the involvement of Th1 cells, the up-regulation of CB2R possibly can modulate the immune response in favor of host and prevent neuro-inflammatory reactions in CNS. Like MS treatment with cannabinoid in HAM/TSP should improve the signs and symptoms and prevent rapid progression.

Keywords: Human T lymphotropic virus type-1, HTLV-1-associated myelopathy/tropical spastic paraparesis, CB1 Receptor, CB2 Receptor

HTLV, a multi organ oncovirus

Samaneh Ramezani[1], Mohammad Mehdi Akbarin[1]

1. Inflammation and Inflammatory Diseases Research Center, Mashhad University of Medical Sciences, Iran

Background: Human T lymphotropic virus (HTLV-I) is a retrovirus that has been recognized as a causative agent of two crucial diseases, HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) and Adult T cell Leukemia-Lymphoma (ATLL).

Methods: The virus not only induces those diseases in a small proportion of HTLV-I carriers (3-5%) but also it is associated with other diseases such as HTLV-I-Associated Arthropathy (HAAP), Cutaneous T Cell Lymphoma (CTCL), Graves' disease, uveitis, polymyositis, chronic respiratory diseases, lymphadenitis and dermatitis. Furthermore, HTLV related and accelerated disorders were more investigated, and the factors that might implicate in the development or progression of diseases have been discussed.

Results: We founded 13 categories of non-associated disease in studies such as Reproductive Disorders, Coronary Artery Disease (CAD), non -ATLL lymphoma, Co-infection, non-HAM/TSP neurological associated disease, non ATLL cutaneous associated disease, Autoimmune-Inflammatory related disease, Kidney disease, Liver disease, Respiratory disease, TB disease and Thyroid disease.

Conclusion: With regard to the reviewed studies suggested HTLV-I disorders can divide into three manifests; related, accelerated and associated disease. However, interaction between HTLV-I infection and host immune response was complicated and vague. Some infectious patients indicated the involvement of inflammatory response of immune system, but in other individuals function of anti-inflammatory elements was observed. For a better understanding of this classification, more systematic studies should be designed and need to provide a global network to control and prevent HTLV affiliated diseases.

Keywords: ATLL; Carriers; HAM/TSP; HTLV-I non-associated disease.

Investigating the causes of vaccination failure against Newcastle Disease and Avian Influenza in Semnan broiler flocks

Khatereh Kafshdouzan[1]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran

Background: The incidence of Newcastle disease (ND) and Avian Influenza (AI) and their economic losses have been documented despite regular vaccination programs. So, it seems imperative to analyze the causes of vaccination failure and find effective ways to prevent and control these diseases.

Methods: In this assay, 11 broiler farms located in Semnan, were randomly investigated since February 2021 to October 2022 in terms of vaccination schedule, incidence of ND and AI and antibody titer against these two diseases.

Results: Among 11 studied herds, 6 of them were diagnosed with AI in terms of clinical symptoms and antibody titers (3 herds after 45 days and 3 other before this age). Regarding Newcastle, 6 out of 11 herds were diagnosed with the disease.

Conclusion: Due to the fact that in all farms, ND and AI bivalent killed virus vaccines were the only ones were used, this conclusion can be drawn that in those units which gotten infected after 45 days, lack of booster dose and insufficient antibody titer caused by killed vaccines have played a role. While in other 3 units, the high severity of the wild strains seems to be the reason of vaccination failure. Considering that the vaccine strains used in the fields are genotype 1 and 2 and the field strain in Iran is genotype 7, the vaccines do not provide sufficient protection. Another reason is the wrong method of vaccination; appropriately the live attenuated booster dose of Newcastle should get inoculated as spray but mostly, for ease of the work, this vaccine is used in drinking water method and this significantly reduces the immunogenicity of the vaccine. Also the proper time of inoculation in such a way that it won't interfere with the maternal antibody does not considered in Newcastle vaccination.

Keywords: Vaccination failure, Newcastle Disease, Avian Influenza

Investigating the Immunosuppressive Effect of Canine Distemper Virus through the Evaluation of Co-infection with Canine Parainfluenza Virus Type 2

Afsaneh Tavakoli Zaniani[1], Azam Mokhtari[1], Mohammad Javad Yazdanpanah[1]

1. Department of Pathobiology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Background: In this study, the aim was to investigate the possibility of co-infection of Canine Distemper Virus (CDV) and Canine Parainfluenza Virus type 2 (CPiV-2) in dogs referred to the veterinary clinics of Isfahan city. CDV is a potent immuno-compromising virus, infecting respiratory, gastrointestinal and nervous system epithelial tissues. Infection with CDV causes lymphocytic infection, leading to an increase in viral load, lymphopenia and subsequent suppression of the host immune system. CPiV-2 is also a very important virus, due to its high transmission and prevalent respiratory infection.

Methods: Respiratory and gastrointestinal specimens were collected from 50 dogs of two groups with clinical and non-clinical manifestations of the Distemper disease, each. Then by using distemper specific immunochromatography detection kits, the presence of the CDV was confirmed as a result of immunological complex formation between pathogenic agents as antigens and specific immunoglobulins produced against the virus as antibodies. After the detection of the virus, RNA extraction and cDNA synthesis were done using extraction and reverse transcription kits. Finally, the samples were evaluated using RT-PCR with both positive and negative pre-prepared control samples and the obtained data were analyzed by SPSS statistical software.

Results: Regarding the results of immunochromatography, 29 and 1 positive cases among dogs suspected to CDV and seemingly healthy dogs, respectively. After performing RT-PCR assay, in symptomatic group, 37 and 11 cases were reported as CDV and CPiV-2 positive, respectively. Furthermore, 3 and 1 cases were determined as CDV and CPiV-2 positive in the asymptomatic group. Moreover, the frequency of CDV and CPiV-2 co-infection was 4%. In addition, all 30 positive cases of immunochromatography results were also positive for RT-PCR assay.

Conclusion: In this study, although immunochromatography results significantly correlated with RT-PCR assay, but no reliable association was found between Canine Distemper Virus and Canine Parainfluenza Virus type 2 infection.

Keywords: Canine Distemper Virus, Canine Parainfluenza Virus, Immunochromatography, Comorbidity

Investigation of IL-2 and IFN-γto EBV peptides in stimulated whole blood among multiple sclerosis patients and healthy individuals

Nastaran Rafiee[1], Mehrdad Ravanshad[1], Bahador Asadi[2], Kianfar Roya[1], Ali Maleki[1]

1. Tarbiat Modares University, Tehran, Iran.
2. Aja University of Medical science

Background: Epstein-Barr virus (EBV) is a double-stranded DNA virus and has two phases of lytic and latent infection in host cells. After infecting B lymphocytes, EBV becomes persistent in these cells. In healthy individuals T lymphocyte has a major role in killing EBV infected B cells. Statistical studies have shown that the risk of MS, is increased in individuals with symptomatic EBV infection. In the present study in order to measure immune response, especially T lymphocytes, against different components of EBV, the mRNA level of IL-2 and IFN-γ, that impress autoimmune diseases and indicate T cell function, has investigated in treated whole blood (WB) culture with EBNA1 and BRLF1 peptides from 10 healthy individuals and 10 MS patients.

Methods: First of all, the viability percent of cells after treating with viral peptides in different period of time was measured by MTT assay. Then optimal annealing temperature for primers function was determined by PCR. Finally, the mRNA level of IL-2 and IFN-γ in treated and untreated WB culture was evaluated by using real time RT-PCR.

Results: The analysis of the results achieved by real time RT-PCR, demonstrated a significant increased level of IL-2 in MS patients than healthy subjects after exposure to both peptides. Also, the mRNA level of IFN-γincreased in MS patients in EBNA1 treated WB culture.

Conclusion: According to obtained results, EBV peptides can reactivate immune cells, especially T lymphocytes, and may indirectly induce inflammation and develop MS; however, it seems that long time exposure to these peptides has reduced effect on T cell function and face the control of B lymphocytes with difficulty.

Keywords: Epstein-Barr virus, Multiple sclerosis, T lymphocyte, B lymphocyte, interferon-gamma, interleukin-2

Investigation of the Immunosuppressive Effect of Canine Distemper Virus through the Evaluation of Co-infection with *Bordetella bronchiseptica*

Mehri Isvand[1], Azam Mokhtari[1], Fatemeh Nasiri-Dashtaki[1]

1. Department of Pathobiology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Background: Canine distemper virus (CDV) is an extremely contagious pathogen. Domestic dogs are the most commonly infected species. *Bordetella bronchiseptica* is a small gram-negative bacterium, responsible for infectious bronchitis in dogs and other animals. Considering the negative effect of CDV on the immune system and increasing the risk of other infections, the present study was conducted with the aim of identifying CDV and investigating the incidence of co-infection with *Bordetella bronchiseptica*, using the genomic and serological methods.

Methods: 50 samples from dogs with the signs of respiratory and digestive tract involvement and 50 samples from apparently healthy dogs referred to Isfahan veterinary clinics were taken. Eye secretions, saliva, urine, and blood samples can be used to diagnose distemper using immunochromatography kits. After the RNA extraction and reverse transcription for CDV detection, and DNA isolation for investigation of *B. bronchiseptica*, PCR was performed. Positive and negative control samples prepared by Isfahan University of Medical Sciences were also included in each test. Finally, positive samples were sent to Bioneer (Korea) for sequencing.

Results: According to the results of the RT-PCR assay, among samples taken from the dogs with suspected CDV infection, 74% were positive for the presence of CDV nucleic acids, and 40% were positive for the presence of B. bronchiseptica nucleic acids. Also, among samples taken from seemingly healthy dogs, 6% were positive for CDV, and 30% were positive for B. bronchiseptica nucleic acids.

Conclusion: In the present study, samples taken from dogs with suspected CDV infection and apparently healthy dogs, a low percentage of cases showed co-infection. Therefore, no significant relationship was found between CDV and B. bronchiseptica infections. It seems that future studies with a larger sample size can provide us with more accurate results.

Keywords: Canine Distemper Virus, *Bordetella bronchiseptica*, Immunochromatography, Co-infection

Investigation of the seroprevalence of anti-measles IgG antibody in students at Shiraz University of Medical Sciences

Mohammad Kia[1], Amir Hossein Alipour[1], Seyed Mohammad Ali Hashemi[1], Fatemeh Nekooei[1], Fatemeh Dashtizadeh[1], Vahid Salimi[2], Mohammad Javad Fattahi[3], Kamran Bagheri Lankarani[4], Jamal Sarvari[5]

1. Department of Bacteriology & Virology, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Virology, Tehran University of Medical Sciences, Tehran, Iran.
3. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Health Policy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
5. Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Measles is a highly contagious, severe illness with a high fatality rate in youngsters. Regardless of the fact that vaccination has reduced measles incidence, outbreaks still occur. We therefore sought to determine the prevalence of anti-measles immunoglobulin G (IgG) antibodies among Shiraz University of Medical Sciences (SUMS) students in this study.

Methods: The participants in this cross-sectional study comprised 450 SUMS students. A questionnaire was used to gather data on demographics and measles vaccination history. Participants were divided into two groups, A and B, based on routine measles vaccination doses and the national measles/rubella (MR) immunization program. Using a commercial ELISA (Enzyme-Linked Immunosorbent Assay) kit, the anti-measles IgG antibodies were assessed.

Results: Ages of participants ranged from 18 to 48, with a mean of 22.2 (±4.3) years. Males and females each made up 50% (225) of the subjects. According to our findings, anti-measles IgG antibodies were present in 63.6% of the patients. Between groups A and B, there was no discernible difference in the seroprevalence of IgG antibodies (p=0.612). The seroprevalence of anti-measles IgG antibodies did not significantly correlate with the subjects' age (p=0.43) or sex (p=0.24).

Conclusion: According to the findings, anti-measles IgG antibodies are not present as frequently as would be necessary to stop the spread of the measles virus. There may therefore be a need for a booster shot.

Keywords: Measles, Anti measles antibody, Immunity, Vaccine

Newcastle disease virus variants synergize the effects of doxorubicin to inhibit tumor growth: evidence from a murine breast cancer model

Somaye Sadeghi[1], Mahdieh Shokrollahi[1], Hamed sabri Sabri[1], Ehsan Zafari[1], Ramin Sarrami-forooshani[1]

1. Advanced Therapy Medicinal Product (ATMP), ATMP Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Iran

Background: Chemoresistance is a major obstacle in cancer treatment. An ideal cancer therapy is achieved the selective killing of the malignant cells, while leaving normal tissues intact. Virotherapy has attracted increasing attention as a potent cancer therapy strategy due to the selective replication in cancer cells, but it seems that the best chance for complete tumor destruction can be achieve by combining its mechanism of action with other treatment strategies. The current study was designed to investigate the possibility of the oncolytic effect induced by Newcastle virus (NDV) and Doxorubicin hydrochloride (Dox) combination in vitro and in vivo.

Methods: The in vitro study include exposure of the different breast cancer cell line (4T1, MDA-MB-231, MC4-L2 and BT-474) to NDV alone or combination with Dox. Animal models of breast received the NDV in combination with Dox. Tumor volume, body weight and survival of the mice were determined throughout the experiment and the toxic effects of the treatments was determined through studying the expression levels of apoptosis-related genes and histopathological studies.

Results: Treatment with NDV reduced cell viability of breast cancer cell lines and increased cell apoptosis. At the end of the experiment, a significant reduction ($p<0.05$) in relative tumor volume was observed in combination therapy groups (NDV+Dox) when compared to the control group. From our data, combinational therapy demonstrated synergistic cytotoxicity.

Conclusion: Our results showed that NDV combined with Dox can suppress tumor development in a dose-dependent manner.

Keywords: Breast cancer, Oncolytic virus, Synergy, Doxorubicin

Relationship between viral load and IL-1α, IL-6, IL-10, and TNF-αcytokines levels with disease severity in Crimean Congo hemorrhagic fever patients

Maryam Rostamtabar[1], Tahmine Jalali[2]

1. Department of immunology, Immunoregulation Research Center, Babol University of Medical Sciences
2. Department of Arboviruses and Viral Hemorrhagic Fevers, Pasteur Institute of Iran

Background: Crimean Congo Hemorrhagic Fever (CCHF) is one of the deadly forms of hemorrhagic fever diseases widely distributed by tick-borne in animals and humans. The recent findings have indicated that cytokines such as IL-1α, IL-6, TNF-α, and IL-10 in response to the CCHF virus (CCHFV) may be implicated in the development of CCHF-viral infection. With regard to the significant role of these cytokines involved in the pathogenesis of CCHF.

Methods: This study aimed to investigate the association of IL-1α, IL-6, TNF-α, and IL-10 cytokines with viral load and severity of the disease in 30 confirm CCHF patients based on the severity of hemorrhagic manifestations in three groups (fatal, severe, and moderate) considered. Infection of CCHF was confirmed by using the detection of CCHFV Genomic RNA by Real-time PCR method and specific IgM, IgG, or both were measured with enzyme-linked immunosorbent assay (ELISA) method.

Results: A remarkable level of TNF-αand IL-10 was observed in fatal cases while moderate cases had the lowest level of these cytokines (p=0.000 and p=0.012). In addition, fatal cases compare to non-fatal cases were the highest level of viral load. In other words, there was a linear positively significant difference in viral load and severity of disease (p=0.000). Keeping in view these results observed a positive linear association between the viral load with IL-10 and TNF-α(p=0.04). In addition, observed a negative linear association was between the viral load with IL-6 and IL-1α level, but there was no significant difference. Moreover, a significant linear negative correlation between the antibody response with viral load. So, IgM antibody response in fatal cases was lower than in the survival-infected patients (p< 0.05). It implies that the high mean viral load correlated with a low level of response to IgM antibodies (p= 0.05).

Conclusion: Our results showed that the severity and hemorrhagic form of the CCHFV may be associated with high levels of IL-10 and TNF- αresponse and viral load. Also, the findings of this study imply that the viral load is closely linked to the severity of infection with CCHFV, which may play a critical role in the immunopathology of CCHF disease.

Keywords: CCHF, IL-1α, IL-6, IL-10, TNF-α

Serosurvey of hepatitis A virus infection among municipal sweepers working in the Shiraz south of Iran

Jamal Sarvari[1], Negar Joharinia[1], Saeed Firoozi Ghahestani[1], Rozita Ghojoghi[1], Kourosh Dorost[1], Abolfazl Gheshlaghi[1], Omid Jaberi[2], Rohollah Khoshbakht[2], Seyed Younes Hosseini[3]

1. Department of Bacteriology & Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Occupational Health Engineering, HSE Unit, Shiraz Waste Management Organization, Shiraz, Iran
3. Department of Bacteriology & Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Hepatitis A virus (HAV) is one of the most common infectious etiologies of acute hepatitis worldwide. The present study was designed to investigate the level of exposure to hepatitis A virus in urban waste collectors/sweepers in Shiraz, southern Iran.

Methods: 385 municipal sweepers of Shiraz city were included in this cross-sectional study. A questionnaire include their demographic and occupational characteristics, as well as their awareness of viral hepatitis disease was fill out. The frequency of IgG against HAV was determined using commercial IgG ELISA kit.

Results: The mean age of participants was 41 ± 8 years. All waste collectors were men. All of 385 (100%) individuals were positive for anti-HAV IgG based on ELISA assay. However, there was no statistical analysis performed for HAV infection and demography and sanitation status, respectively.

Conclusion: Our finding showed a slightly higher frequency of anti IgG Ab against HAV in municipal sweepers working in comparison with other population therefore municipal sweepers working might not be a risk factor for HAV infection. More study is recommended for verify this finding.

Keywords: Hepatitis A virus, Street sweeper, Serology, Iran

Serosurvey of hepatitis E virus infection among municipal sweepers working in the largest city in the south of Iran

Seyed Younes Hosseini[1], Negar Joharinia[1], Seyed Ali Hosseini[1], Saeed Firoozi Ghahestani[1], Rozita Ghojoghi[1], Abolfazl Gheshlaghi[1], Omid Jaberi[2], Rohollah Khoshbakht[2], Jamal Sarvari[3]

1. Department of Bacteriology & Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Occupational Health Engineering, HSE Unit, Shiraz Waste Management Organization, Shiraz, Iran
3. Department of Bacteriology & Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Among viral hepatitis, Hepatitis E virus (HEV) is one of common causes of viral hepatitis worldwide. The present study was designed with the aim of determining the level of exposure to hepatitis E viruses in urban waste collectors/sweepers in southern Iran.

Methods: 385 serum samples were collected from municipal sweepers of Shiraz city. A questionnaire about their demographic and occupational characteristics, as well as their awareness of viral hepatitis disease information was collected. The frequency of anti IgG Ab against HEV was determined using commercial IgG ELISA kit.

Results: All participants were male, with the mean age of 41 ± 8 years. Out of 385 individuals 62 subjects were positive for anti-HEV IgG based on ELISA assay. Also, the frequency of HEV IgG antibody among age groups 20–30, 31–40, 41–50 and >50 years old had an increasing trend, 4.5%, 10.1%, 17.4%, and 36.7%, respectively, indicating age factor significance (p=0.001). There was no statistically significant difference between anti-HEV IgG positive versus negative sweepers based on some other occupational characteristics.

Conclusion: According to the results collecting/sweeping of garbage might not be a risk factor for HEV infection. More study is recommended for verify this finding.

Keywords: Hepatitis E virus, Street sweeper, Serology, Iran

The frequency of IgG anti-rubella antibody in female students of Shiraz University of Medical Sciences

Amir Hossein Alipour[1], Seyed Mohammad Ali Hashemi[1], Saghar Maleki[1], Fatemeh Nekooei[1], Fatemeh Dashtizadeh[1], Gholam Reza Daryabor[2], Kourosh Kalantar[3], Vahid Salimi[4], Jamal Sarvari[1]

1. Department of Bacteriology & Virology, Shiraz University of Medical Sciences, Shiraz, Iran
2. Autoimmune Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Virology, Tehran University of Medical Sciences, Tehran, Iran

Background: It is possible to determine the biological risk associated with the exposure of high-risk groups to the rubella viruses by evaluating one's immunity to those diseases. Determining the prevalence of IgG anti-rubella antibody (Ab) in female students at Shiraz University of Medical Sciences (SUMS) was our goal as a result.

Methods: 434 female students in all were involved in the study. Sera were extracted from blood samples and kept at -20°C for later analysis. A record of the questionnaire form, which asked for demographic data and a history of vaccinations, was made. Students who had enrolled were separated into recipients of either 2 or 3 doses of the measles/rubella (MR) vaccine. Using commercial IgG immunoassays, serum samples were examined for IgG Ab against rubella virus.

Results: Students were 21.6±4.25 years old on average. The findings revealed that 287 (66.1%) of the students had anti-rubella IgG-Ab. Those who got three doses of the MR vaccination had a considerably increased frequency of anti-rubella IgG Ab (P<0.001).

Conclusion: According to our findings, those who had only gotten two doses of the rubella vaccine may need a third dosage.

Keywords: Rubella, Anti rubella antibody, Immunity, Vaccine

The frequency of IgG anti-varicella antibody in female students at Shiraz University of Medical Sciences

Seyed Mohammad Ali Hashemi[1], Amir Hossein Alipour[1], Zinab Khatab[1], Fatemeh Nekooei[1], Fatemeh Dashtizadeh[1], Gholam Reza Daryabor[2], Kourosh Kalantar[3], Vahid Salimi[4], Jamal Sarvari[1]

1. Department of Bacteriology & Virology, Shiraz University of Medical Sciences, Shiraz, Iran
2. Autoimmune Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Virology, Tehran University of Medical Sciences, Tehran, Iran

Background: Patients who are vulnerable, notably children and people with impaired immune systems, are at risk of contracting varicella in hospitals in addition to healthcare personnel who are not immune to the virus. Testing for immunity to varicella-zoster viruses (VZV) can serve as a biological risk indicator for their exposure in high-risk populations. Thus, our goal was to assess the prevalence of IgG anti-varicella antibody (Ab) in female students at Shiraz University of Medical Sciences (SUMS).

Methods: In this study, a total of 434 female students were included. Sera from blood samples were isolated and stored at -20°C until further analysis. The questionnaire form, including demographic information and vaccination history, was recorded. Enrolled students were divided into recipients of either 2 or 3 doses of the measles/rubella (MR) vaccine. Serum samples were tested for varicella zoster virus IgG Ab using commercial IgG immunoassays.

Results: Participants' ages ranged from 18 to 48, with a mean age of 21.6±4.25. Serological testing revealed that 292 students (67.3%) had anti-varicella IgG Ab.

Conclusion: Therefore, we suggest adding the VZV vaccine to Iran's regular immunization schedule since nearly 40% of female students were vulnerable to VZV infection. These findings need to be confirmed by other research.

Keywords: Varicella, Anti varicella antibody, Vaccine

The sero prevalence of hepatitis B in municipal waste collectors in Shiraz, southwest of Iran

Jamal Sarvari[1], Saeed Firoozi Ghahestani[1], Negar Joharinia[1], Alireza Shiri[1], Atefeh Vali[2], Mohammad Ali Davarpanah[3], Amir Atapour[4], Omid Jaberi[5], Seyed Younes Hosseini[1]

1. Department of Bacteriology & Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Shiraz HIV/AIDS Research Center, Institute of
4. Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz
5. Health, Safety and Environment Unit, Shiraz Waste Management Organization, Shiraz, Iran

Background: Chronic HBV infection remain a main health problem in the world. This study conducted to determine the frequency of hepatitis B virus (HBV) serological markers among waste collectors in the southwest of Iran.

Methods: 385 blood samples were collected from waste collectors in the 10 districts of municipality of Shiraz city. A questionnaire including occupational and demographic information as well as awareness about viral hepatitis form were gathered from all participant. The presence of hepatitis B surface antigen (HBsAg) and anti-HBs antibody were assayed using ELISA commercial kits.

Results: All participants were male, with the mean age of 41 ± 8 years. Out of 385 sera, 38 (9.9%) samples had a protective level of anti HBs antibodies, while more than 90% had a low level of anti HBs antibodies. Also, 6 (1.5%) subjects were positive for HBsAg participant's antibodies.

Conclusion: Our findings indicated that this occupation might not be a risk factor for the acquisition of HBV, but evaluation of HBsAg and anti-HBs levels should be a priority in the healthy program of waste collector workers (WCWs).

Keywords: HBV, prevalence, waste collector worker, Iran

The sero prevalence of hepatitis C viruses in municipal waste collectors in Shiraz, Iran

Saeed Firoozi Ghahestani[1], Jamal Sarvari[1], Negar Joharinia[1], Alireza Shiri[1], Farshid Beigzadeh[1], Rohollah Khoshbakht[2], Omid Jaberi[2], Seyed Younes Hosseini[1]

1. Department of Bacteriology & Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Health, Safety and Environment Unit, Shiraz Waste Management Organization, Shiraz, Iran

Background: Hepatitis C virus (HCV) still remains as a challenging health issue worldwide. This study conducted to determine the frequency of HCV serological markers among waste collectors in the southwest of Iran.

Methods: In this cross-sectional study, 385 waste collectors in the 10 districts of municipality of Shiraz were included. A questionnaire including occupational and demographic information as well as awareness about viral hepatitis were gathered from participants before sampling. The presence of anti-HCV antibody was assay using ELISA assay.

Results: The mean age of participants was 41 ± 8 years. All participants were male. The results of ELISA assay showed that all participants (100%) were negative for HCV antibodies. However, the majority (>79%) of individuals significantly were not aware of the risk of blood transmission route of the hepatitis-related virus.

Conclusion: Based on our findings this occupation might not be a risk factor for the acquisition of HCV infections, but evaluation of anti HCV levels should be a priority in the healthy program of waste collector workers (WCWs).

Keywords: HCV, prevalence, waste collector worker, Iran

Weighted gene co-expression network analysis reveals candidate modules and important hub-high traffic genes involved in human respiratory syncytial virus infection

Aliakbar Hasankhani[1], Abolfazl Bahrami[1]

1. Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Background: Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infection and mortality in children. Due to limited treatment options for children and insufficient knowledge of the interaction between host and pathogen, this study was performed to investigate the molecular mechanisms of the immune system involved in RSV infection and identify therapeutic targets using the systems biology approaches.

Methods: Publicly available RNA-Seq data from whole blood of 10 healthy and 31 RSV infected children were downloaded from the Gene Expression Omnibus (GEO) and then analyzed to generate gene expression matrix. Based on the assumption that the connectivity patterns and network density of the non-preserved module changes under RSV infection, these modules may be suitable candidates for identifying the mechanisms and biological pathways involved in the disease. Therefore, weighted gene co-expression network (WGCNA) and functional enrichment analysis was used to identify (1) non-preserved modules between healthy (as reference set) and RSV (as test set) infected samples and (2) RSV biologically related modules, respectively. Furthermore, protein-protein interaction (PPI) networks based on the co-expressed hub genes from candidate modules were extracted and high traffic genes were then identified based on high values of betweenness centrality (BC).

Results: Using WGCNA, 37 modules were identified in healthy samples as reference set. In agreement with our hypothesis, among the identified modules, the topological structure of 32 modules were changed under RSV infection. The results of functional enrichment analysis showed that among 32 non-preserved modules, six modules including purple, green, brown, salmon, saddlebrown, and pink were highly related to host immune response and RSV pathogenesis. Some important KEGG pathways and biological processes in the six candidate non-preserved modules was included “type I interferon signaling pathway”, “NOD-like receptor signaling pathway”, “Phagosome”, “B cell receptor signaling pathway”, “neutrophil mediated immunity”, “T cell receptor signaling pathway”, and “interferon-gamma-mediated signaling pathway”. We also identified a set of hub-high traffic genes, including STAT1, DDX58, MX1, IRF7, ISG15, SOCS3, IRF8, TLR7, IFI35, IFIH1, DHX58, RSAD2, SOCS2, NOD1, GZMB, IRAK1, LYN, IRF1, TLR4, and IFIT3 that play a critical role in the host immune response during RSV infection.

Conclusion: Using computational methods, this study helps us to better understanding the molecular mechanisms during RSV infection. Moreover, this study also suggests several candidate modules and important genes as therapeutic targets for inhibition of RSV infection development.

Keywords: respiratory syncytial virus, weighted gene co-expression network, protein-protein interaction, RNA-seq, hub-high traffic genes, betweenness centrality.

Immunosenescence and Aging

CD153+ activated monocytes increase in the peripheral blood of patients with Buerger's disease

Niloofar Mashhadi[1], Leila Kasraian[2], Hamed Ghoddusi Johari[3], Ahmad Hosseinzadeh[3], Nasim Kheshtchin[1], Mehrnoosh Doroudchi[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Blood Transfusion Research Center, High Institute for Education and Research, Shiraz, Iran
3. Department of Surgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Monocytes secrete various inflammatory cytokines such as TNF-α, IL-1βand IL-6 upon activation and monocyte-induced inflammation is associated with immune aging. Inflammation can further induce immunosenescence in monocytes which then can interact and affect endothelial cells. Buerger's disease or Thromboangiitis Obliterans (TAO) is an inflammatory disease of peripheral vessels in which the exact pathological mechanisms remain unknown. We investigated the expression of CD57 and CD153 senescence markers on monocytes in peripheral blood of patients with TAO.

Methods: Our case-control study comprised of 9 smoker men with advanced TAO, 9 healthy smoker men and 9 healthy non-smoker men. The percentages of senescent peripheral monocytes were detected by flow-cytometry using CD3 exclusion gating. The results were analyzed using non-parametric statistical tests.

Results: The frequency of CD3- monocytes significantly decreased in patients with TAO and healthy smokers compared to healthy non-smoker individuals ($p<0.001$ and $p=0.018$, respectively). The frequency of CD3-CD57-CD153+ monocyte was significantly higher in patients in comparison with healthy non-smoker individuals ($p= 0.026$).

Conclusion: The higher frequency of CD153 expressing monocytes in patients suggests that patients' monocytes are at a state of activation and perhaps play a role in the Buerger's disease through their inflammatory phenotype.

Keywords: Thromboangiitis Obliterans, Inflammation, Immunosenescence, CD57, CD153/CD30L

CD3+CD4-CD153+ lymphocytes increase in the peripheral blood of patients with Buerger's disease

Niloofar Mashhadi[1], Leila Kasraian[2], Hamed Ghoddusi Johari[3], Ahmad Hosseinzadeh[3], Nasim Kheshtchin[1], Mehrnoosh Doroudchi[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Blood Transfusion Research Center, High Institute for Education and Research, Shiraz, Iran
3. Department of Surgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Inflammatory mediators produced by senescent immune cells promote the inflammatory microenvironment in blood vessels that leads to various cardiovascular diseases. Thromboangiitis Obliterans (TAO), also called Buerger's disease, is a systemic vasculitis which causes several segmental occlusions particularly in young smoker men. We assessed the expression of CD57 and CD153 (CD30L) as senescence markers and evaluated the frequency of senescent CD3+CD4- lymphocytes (assumingly CD8+ T cells) in patients with TAO.

Methods: Study subjects included 9 non-atherosclerotic, non-diabetic male smoker patients with TAO as case group and 9 healthy smokers and 9 healthy non-smokers as control groups. Case and control groups were sex-matched. The percentages of senescent CD4- T cells were detected by flow-cytometry. The results were analyzed using non-parametric statistical tests.

Results: The frequency of senescent CD3+CD4-CD57-CD153+ T cells was higher in patients compared to smoker controls (P = 0.02). In patients with TAO, CD57+CD153- cells were more frequent in CD3hiCD4-T cells compared to CD3loCD4- T cells (p=0.008). Conversely, the frequency of CD57-CD153+ T cells were significantly higher in CD3loCD4- T cells compared to CD3hiCD4- T cells (p= 0.004).

Conclusion: The higher frequency of senescent CD3+CD4-CD57-CD153+ T cells as well as higher T cells activation rate (CD3lo population) in patients implies that senescent CD4- T cells have an important role in TAO development.

Keywords: Thromboangiitis Obliterans, CD57, CD153/CD30L, Cigarette Smoking, Immunosenescence

Comparison of the activity of Macrophages derived from monocytes obtained from leukocyte reduction filters in the aging process

Afrooz Homayoonfar[1]

1. Tarbiat Modares University, Tehran, Iran.

Background: Aging is a progressive process with physiological changes. In aging process, the body's immune system's ability to fight infections, tumors and respond to vaccinations is reduced, and on the other hand, it is prone to autoimmune diseases. The important and undeniable place of macrophages in the human immune system has made researchers interested in studying these cells as much as possible. One of the oldest and most common sources for providing cells for studies and research is the use of standard buffy coats obtained from blood transfusion organizations. Due to the negative effects of the presence of leukocytes in blood products, the policy of blood transfusion medicine has been to use several methods to remove leukocytes, the most effective of which is the filtration of donated blood before storage.

Methods: In this study, we aimed to extract cells trapped in leukocyte-reducing filters (LRF) without cell damage with emphasis on monocyte isolation, and after evaluating cell viability, differentiated monocytes into macrophages.

Results: Investigate the aging process on the expression of surface markers and the function of these cells. For this purpose, age groups of 20 to 60 years were used for comparison.

Conclusion: Comparing the data obtained from the forthcoming study with the reports provided by the studies of others, the results of phenotypic and functional study of and macrophages in the aging process agree with some studies and in contrast to some other reports indicate the need for more detailed study of the effect of aging on cellular mechanism, signaling and the presence of dendritic cells and macrophages in It has an immune system.

Keywords: Aging, Immunosenescence, Macrophages

Development of electrochemical nanobiosensor for early diagnosis of Alzheimer's disease

Forough Chakari-khiavi[1], Mohammad-Reza Rashidi[2], Mehdi Yousefi[2], Balal Khalilzadeh[3], Leili Aghebati-Maleki[4], Koorosh Shahpasand[5]

1. *Department of Medicinal Chemistry, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*
2. *Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Stem Cell Research Center (SCRC), Tabriz University of Medical Sciences, Tabriz, Iran*
4. *Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
5. *Cancer Research Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston*

Background: Alzheimer's disease is a common severe neurodegenerative disorder characterized by cognitive disorders and memory loss in different stages and in severe cases this disease could be evolved to dementia and even death. The late onset of clinical symptoms in Alzheimer's patients is one of the main reasons why drug therapy is delayed and generally causes failure in the treatment of Alzheimer's patients. Nowadays, studies on the pathogenesis of this disease, which mainly includes tau protein (total and phosphorylated forms (p-tau)) and amyloid-b peptides (Ab1-40 and Ab1-42 isoforms), makes this possible to properly monitor the progression of Alzheimer's disease. So, early stage, sensitive and selective detection of this silent disease is highly needed in biomedical research area.

Methods: In this study, an electrochemical immunosensor was developed for specific detection of Alzheimer's disease using cis-tau protein as biorecognition element. ZIF-Pt based nanocomposite was applied as conductive and high surface area platform, which was drop-cast on the glassy carbon electrode (GCE). Then, Antibody of cis-tau protein was covalently immobilized on the modified GCE via EDC/NHS coupling chemistry. Finally, the target protein was sensitively captured by the designed immunosensor. Different electrochemical techniques were applied for evaluation of the developed immunosensor. Also, the modification steps and the electrode morphology were characterized by SEM and EDX. After passing all optimization steps, the acceptable detection limit of 10-15M and linear dynamic range of 10-8-10-15 M were obtained, respectively.

Results: A new label-free electrochemical immunosensor is reported for selective and sensitive detection of cis-tau as an AD biomarker. Under the optimized experimental conditions, the proposed immunosensor exhibited strong analytical performance for cis-tau detection in standard and human serum samples in a linear range of 1 fg mL-1 to 10 ng mL-1 with a low detection limit of 1fgmL-1.

Conclusions: As a proof of concept, the designed immunosensor was desirably applied for evaluation of cis-tau in different Alzheimer's suffering patients at different stages. Consequently, the prepared biosensor is capable to use as reference test in biomedical laboratories.

Keywords: Biosensor, immunosensor, electrochemical, Alzheimer's disease, Tau protein

LDLR rs2228671 CC genotype is associated with lower BMI in men with diabetes mellitus

Afsaneh Zare[1], Pegah Pourzargham[1], Atefe Ghamar Talepoor[1], Mehrnoosh Doroudchi[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Type 2 Diabetes Mellitus (T2DM) is a chronic and age–related inflammatory disease. Although cardiovascular complications in patients with T2DM are common, the exact relation between DM and Cardiovascular Diseases is still unknown. LDL receptor (LDLR) is responsible for regulating plasma LDL-cholesterol concentrations and can associate with Insulin receptor on the cell membrane. This binding decreases the LDL particle clearance and can be modulated by the insulin level. Among LDLR polymorphisms, rs2228671 single nucleotide polymorphism (SNP) (C>T) has shown the strongest association with LDL-cholesterol level. In this study we evaluated the genetic link between LDLR and progression of atherosclerosis in diabetic and non-diabetic individuals.

Methods: Our study was conducted on 485 individuals who resided in Fars province of Iran. Based on the Diagnostic Angiography, patients were categorized to diabetes+angio+ (n=63), diabetes+angio- (n=48), and diabetes-angio+ (n=118) groups. Also 256 healthy blood donors were recruited and considered as control group (diabetes-angio-). DNA was extracted from peripheral blood by salting out method and LDLR gene polymorphism was detected by RFLP- PCR.

Results: We did not observe any significant difference in LDLR rs2228671 genotypes and alleles between the four groups. However, comparison of the 3 patients' groups by regression analysis showed that CC genotype was increased significantly in men who had BMI lower than 25 (P=0.043). Accordingly, the frequencies of men and women with BMI<25 and CC genotype were 80% and 20%, respectively. While the frequencies of men and women with BMI>25 and CC genotype were 57.6% and 42.4%, respectively. There was also a significant difference between the 3 patients groups based on smoking status (p=0.005), gender (p=0.003) and blood pressure (P=0.001). Only 10.3% of smokers were diabetes+angio+, but 79.5% of smokers were diabetes-angio+. While 47.8% of female patients were diabetes+angio+, 67.9% of male patients were diabetes-angio+. Of those patients who had hypertension 45.9% were diabetes+angio+ while 76.8% of those without hypertension were diabetes-angio+.

Conclusion: We suggest that genetic variations in LDL clearance pathways may affect the progression of atherosclerosis in diabetic patients. The association of CC genotype with lower BMI may also have a protective role against diabetes in men. Also, hypertension plays a more significant role than smoking in progression of atherosclerosis in diabetic patients.

Keywords: LDLR, Type 2 Diabetes Mellitus, Angiography, hypertension

Partial recovery of senescence in Circulating Follicular Helper T Cells after Dasatinib treatment

Atefe Ghamar Talepoor[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Cellular senescence is an irreversible cell cycle arrest triggered by different stimuli, including DNA damage, telomere shortening and oncogenic stress. Senescent cells, by releasing the senescence-associated-secretory-phenotype (SASP), contribute to various diseases pathogenesis. Human atherosclerotic plaque contains cells with multiple markers of senescence that associate with disease severity. Therefore, we assessed the frequency of senescent CD3+CD4+CXCR5+CD57+CD153+ cTfh cells as well as senescence gene expression before and after treatment with Dasatinib in patients with low stenosis (stenosis <50%) and high stenosis (stenosis ≥ 50%).

Methods: Twelve high (≥ 50%), and twelve low (<50%) stenosis patients and six healthy controls were enrolled. PBMCs of participants treated with 50 nM and 100 nM of Dasatinib and after 18 h incubation, the cells were used for the flow- cytometric analysis and RNA extraction.

Results: The percentage of senescent CD3+CD4+CXCR5+CD153+CD57+ cells was significantly decreased in Dasatinib treated cells from individuals with low and high stenosis (p=0.0007 and p=0.0002, respectively). However, the frequency of total lymphocytes, CD3+ and CD4+ T cells were not significantly different between the groups before and after treatment. The expression levels of P53 (p=0.0003 and p=0.0001), P16 (P=0.0005 and P=0.0002), p21 (P=0.0002 and p<0.0001), SENEX (p=0.0005 and p<0.0001) and BCL-2 (p=0.0005 and p=0.0002) were decreased in PBMCs of low and high stenosis groups after treatment with Dasatinib, respectively. The percentage of senescent cTfh cells positively correlated with cholesterol (p=0.034; r=0.671), C-reactive protein (CRP) (p=0.029; r=0.707), Erythrocyte sedimentation rate (ESR) levels (p=0.030; r=0.598) and neutrophil counts (p=0.021; r=0.799) in patients with high stenosis.

Conclusion: The decreased frequency of senescent cTfh cells and the expression levels of senescence genes after Dasatinib treatment in patients with atherosclerosis suggest a role for Dasatinib in partial clearance or rejuvenation of senescent cTfh cells, which may decrease inflammatory mediators and attenuate disease progression.

Keywords: "Cellular senescence", "Atherosclerosis", "circulating T follicular helper", "Dasatinib", "Senolytic drug"

Rs2295080 single nucleotide polymorphism of mTOR in diabetic patients with and without atherosclerosis

Afsaneh zare[1], Gholamreza Daryabor[2], Shahdad Khosropanah[3], Mehrnoosh Doroudchi[3]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Autoimmune Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Cardiovascular Research Center, Shiraz University of Medical sciences, Shiraz, Iran

Background: Atherosclerosis is one of the life-threatening consequences of type 2 diabetes mellitus that increased dramatically with age. As a pivotal part of diverse signaling pathways associated with the inflammatory responses, mammalian Target of Rapamycin (mTOR) is an intriguing link between these two diseases. The rs2295080 single nucleotide polymorphism (SNP) of mTOR gene (G>T) is strongly associated with several inflammatory diseases in Asia such as different cancers and coronary artery disease (CAD). This SNP is placed in the promoter region and affects mRNA transcription of the gene. In this study we investigated the mTOR rs2295080 SNP in patients with diabetes and atherosclerosis in a sample of southwest Iranians.

Methods: Altogether 411 men who were residents of Fars province were recruited to this study and were divided to 4 groups based on their Angiography results and Diabetes status. 26 patients as diabetes+angio-, 31 patients as diabetes+angio+, 96 patients as diabetes-angio+. Also 258 healthy blood donors (All men) were recruited and considered as control group (diabetes-angio-). DNA was extracted from peripheral blood by salting out method and mTOR gene polymorphism was detected by RFLP- PCR.

Results: Multinomial regression analysis showed a significant lower frequency of patients with hypertension in diabetes-angio+ group as compared to the diabetes+angio+ (6.8% vs. 80.7%, p= 0.001) group, while there was a higher frequency of smokers in diabetes-angio+ compared to diabetes+angio+ (82.8% vs. 10.9%, p= 0.03) group. We did not find any significant difference in the allele and genotype frequencies of mTOR rs2295080 SNP between the 4 groups.

Conclusion: Lack of difference in the frequencies of genotypes and alleles of rs2295080 SNP in our study is in contrast to the protective role of G allele and GG genotype of this SNP against different cancers. The finding that hypertension but not smoking is a more important factor in the progression of atherosclerosis to clinically significant atherosclerosis is noteworthy and needs further investigation.

Keywords: Type 2 Diabetes, Atherosclerosis, mammalian Target of Rapamycin, Single nucleotide polymorphism

Senescent CD4+T lymphocytes increase in the peripheral blood of patients with Buerger's disease

Niloofar Mashhadi[1], Leila Kasraian[2], Hamed Ghoddusi Johari[3], Ahmad Hosseinzadeh[3], Nasim Kheshtchin[1], Mehrnoosh Doroudchi[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Blood Transfusion Research Center, High Institute for Education and Research, Shiraz, Iran
3. Department of Surgery, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Accumulation of senescent cells can cause various inflammatory diseases of the cardiovascular system through the secretion of senescence-associated secretory phenotype (SASP). Immunosenescence is not only the cause of inflammation, but also occurs as the results of it. Thromboangiitis Obliterans (TAO) or Buerger's disease is an inflammatory obstructive vascular disease of unknown etiology, which is strongly associated with cigarette smoking and mostly affects men. The objective of this cross-sectional case-control study was to assess the frequency of senescent CD4+ T cells expressing CD57 and/or CD153 (CD30L) in patients with TAO.

Method: Nine non-atherosclerotic, non-diabetic, smoker men with advanced TAO, nine healthy smoker men and nine healthy non-smoker men were included in this study. The percentages of senescent CD4+ T cells were detected by flow-cytometry.

Results: Analysis of the senescent CD3+CD4+CD57+CD153+ and CD3+CD4+CD57-CD153+ T cells frequencies showed significant increases in patients compared to healthy non-smoker individuals ($P = 0.01$ and $P = 0.04$, respectively). However, the Median Fluorescent Intensities of CD57 and CD153 were lower on CD3+CD4+CD57+CD153+ T cell subset in patients compared to both control groups (p=0.04 and p=0.01, respectively). The percentage of CD3+CD4+CD57+CD153- T cells negatively correlated with smoking level in smoker controls (p= 0.02, r = -0.80).

Conclusion: The higher frequencies of senescent CD3+CD4+CD57+CD153+ and CD3+CD4+CD57-CD153+ T cells in patients compared to healthy non-smoker controls showed that immunosenescence may have a role in progression of TAO. The Lower expression levels of CD57 and CD153 on CD4+CD57+CD153+ T cells in patients along with higher frequency of CD4+CD57+CD153+ T cells in patients may indicate a possible shift from CD57-CD153+ cells to CD57+CD153+ and finally to CD57+CD153- T cells. This is important because TAO mostly affects young and middle-aged individuals in whom we generally do not expect senescence to be an issue.

Keywords: Thromboangiitis Obliterans, CD57, CD153/CD30L, Cigarette Smoking, Immunosenescence

Immunomodulation and Immunoregulation

Autoimmunity and Schizophrenia

Marziyeh Soltani[1], Nader Bagheri[2], Pezhman Beshkar[3], Kobra Mokhtarian[4]

1. Students Research Committee, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
2. Department of Immunology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
3. Department of Laboratory Sciences, School of Allied Medical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran

Background: The alpha 7 nicotinic acetylcholine receptor (α7AChR) is an ion channel involved in the auditory gate. Disturbance in the signaling of this channel can lead to auditory hallucinations. Therefore, our hypothesis is that the disorder of the immune system and the production of autoantibodies against this channel may lead to auditory hallucinations in patients with Schizophrenia (SCZ). The purpose of this study was to investigate the role of autoantibodies against α7AChR in patients with SCZ.

Methods: A total of 40 patients with SCZ and 40 healthy subjects were participated in this study. Antibodies against α7AChR in patients with SCZ were measured using the indirect immunofluorescence assay and analyzed in ImageJ program. The chi-square ($\chi 2$) test or Fisher's exact test were used to compare the frequency of autoantibodies between patients with SCZ and healthy subjects.

Results: Among 40 SCZ patients, none of the patients showed IgG antibodies against α7AChR. In fact, IgG antibody against α7AChR was not observed in the patient and healthy subjects and there was no difference.

Conclusion: Autoantibodies against α7AChR not exist in patients with SCZ. Although our study did not show any difference between the two groups, we suggested to investigate this autoantibody in a larger statistical population and with different method. Because of the presence of auditory hallucinations in schizophrenic patients and the main role of this channel in auditory gates should be given more attention and investigation.

Keywords: Schizophrenia, Immune system, Autoantibodies, alpha 7 nicotinic acetylcholine receptor, Pathophysiology

Characterization of IL-23 Ss-DNA Aptamer and Inhibitory Effects of It on MCF-7 Cell Line Proliferation

MohammadAli Rezaee[1], Mojtaba Sankian[1], Saeideh Sadat Shobeiri[1], Navideh Haghnnavaz[1], Safoora Pordel[1]

1. Mashhad University of Medical Sceinces, Mashhad, Iran.

Background: IL-23 is the key cytokine in psoriasis and Crohn's disease pathogenesis. Inhibitory agents like antibodies against IL-23 have a good effect in treatment of these diseases. The aim of this study is characterization ss-DNA aptamer against IL-23 and then evaluate the inhibitory effects of it on cell proliferation of MCF-7 cell line (IL-23 receptor positive) in vitro.

Methods: P19 subunit of IL-23 was expressed and purified with affinity chromatography. Systematic evolution of ligands by exponential enrichment (SELEX) method was used for characterizations of ss-DNA against P19 from 1014 ss-DNA library. Aptamer binding to P19 was evaluate using dot blot. Aptamers that have a good binding to P19 were cloned and sequenced and after truncation of them. Different concentration of synthesized aptamers were used to treat MCF-7. Cell proliferation were evaluate using MTT assay.

Results: Sequencing and truncation of characterized aptamer against IL-23 showed 5 specific aptamers have high affinity to P19. Three aptamers decreased cell proliferation in 25 and 50 mM compared to untreated cell group.

Conclusion: Our findings showed three specific ss-DNA aptamers bind to IL-23 with high affinity and attenuated cell proliferation of MCF-7 cell line.

Keywords: IL-23, Aptamer, MTT assay, SELEX, Cell proliferation.

Comparison between the Immunomodulatory Effects of the Naringenin and Prednisolone

Fatemeh keivan[1]

1. Urmia university of Medical Sceices, Urmia, Iran.

Background: Naringenin is a naturally occurring flavonoid found in grapefruit and other citrus fruits. The effect of one-month oral administration of naringenin (10, 20, and 40 mg/kg) or prednisolone (2 mg/kg) on peritoneal macrophage function was compared in the first set of experiments. Separate evaluations were conducted on the effects of naringenin onin vivo and ex vivo T helper (Th) lymphocyte responses and their subsets in mice immunized with ovalbumin (OVA).

Methods: Animals challenged with OVA received varying oral doses of naringenin or prednisolone from two days prior to immunization to 28 days after immunization. The administration of naringenin or prednisolone increased macrophages' respiratory burst, nitric oxide, and IL-10 production while decreasing their IL-12 production. Macrophages isolated from rats administered 40 mg/kg naringenin had greater phagocytic potential than those isolated from rats administered prednisolone. In vivo results revealed that OVA-challenged rats treated with 40 mg/kg naringenin or prednisolone had decreased delayed-type hypersensitivity comparable to control mice. The splenocyte proliferation index was lower in the prednisolone-treated group than in the naringenin-treated group, even at 40 mg/kg.

Results: In the splenocyte cultures, both agents decreased T-bet expression but increased the expression of FOXP3. Naringenin, in contrast to prednisolone, did not affect GATA3 expression. The 40 mg/kg naringenin dose reduced RORγt more effectively than prednisolone.

Conclusion: As naringenin inhibited antigen-specific lymphocyte proliferation less than prednisolone and was incapable of altering the level of Th2 responses, this indicated its potential to act as an immunomodulator as opposed to prednisolone's immunosuppressive properties.

Keywords: Naringenin; Immunization; Immunomodulation; Immunosuppression.

Comparison of the CD4+ CD25+ CD39+/- T Cells Frequency and Expression Level of Deltex-1 Gene in Kidney Transplant Recipients with Normal Long Term Graft Function and Chronic Graft Dysfunction

Kimiya Rahimifard[1]

1. Babol University of Medical Sciences, Babil, Iran.

Background: The effect of regulatory T (Treg) cells on limiting inflammatory responses has been proven. However, subpopulations of this cell have different powers in suppressing alloreactive responses. Therefore, more accurate knowledge of Treg cells, which positively affect transplant survival, can be beneficial for future therapeutic purposes. The primary purpose of this study was to evaluate the frequency of Treg CD39+ CD73+ cells and the expression level of Deltex-1 gene on long-term kidney transplant function.

Methods: The 49 participants in the study were divided in to three groups: Excellent long-term graft function (ELTGF), chronic graft dysfunction (CGD) and Healthy control (HC). Blood sample was collected and PBMC was isolated. Then after performing the relevant steps, the expression level of Deltex-1 gene and the frequency of CD4+ FOXP3+ CD39+ CD73+ T cells were examined.

Results: the data from this study showed that although there is no significant difference in the frequency of regulatory T lymphocytes between the two groups of transplant recipients, but the frequency of CD4+ FOXP3+ CD39+ CD73+ T cells and the ratio of these cells to total CD4+ lymphocytes in the group ELTGF was significantly higher than the CGD group. Also, the expression level of Deltex-1 gene in the CGD group showed a significant decrease compared to the other two groups. There was also an inverse relationship between the the expression level of this gene and the creatinine level in transplant recipients.

Conclusion: Based on these findings, we can conclude that indicators related to the activity of Treg cells play an essential role in maintaining tolerance and quality of transplant function in the long-term.

Keywords: Kidney transplantation, Treg, CD39, CD73, Deltex-1

Correlation of MIR-595 Expression in PBMCs of Patients with Coronary Artery Disease in Comparison to the Control Group

Mohammad Hossein Etemadi[1], Shakiba Kazemian[1], Nader Bagheri[2]

1. Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran
2. Department of Microbiology and Immunology, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

Background: In cardiovascular disease (CAD), inflammation can play an important role in pathogenesis and progress of disease. Inflammatory reaction could lead to vascular endothelial cell damage and plaque formation in atherosclerosis. MicroRNAs (miRNAs), by regulating essential gens post-transcriptionally, may have role to prevent atherosclerotic lesions. This study aimed to evaluate the expression of MIR-595 in peripheral blood mononuclear cells (PBMCs) of patients with CAD in comparison to the control group and its correlation to the number of carotid artery stenosis (CS) in the CAD group.

Methods: In this cross-sectional study, 168 Iranian participants, which include 84 CAD and 84 control, were examined in Hajar Hospital in shahrekord, Chaharmahal and Bakhtiari province, Iran. All patients' anthropometric data such as BMI, systolic and diastolic blood pressure were collected. Expression levels of miR-595 was measured using real-time PCR technique.

Results: A comparison of the CAD group with the control group indicated upregulation of miR-595 (FC=1.9, p=0.009) and expression of miR-595 was considerably enhanced in group CS3 compared to other groups (CS2 and CS1).

Conclusion: Progression of CAD may be affected by upregulated expression of miR-595 and it may be increased the risk of clogged arteries.

Keywords: MIR-595, Cardiovascular disease, Inflammation

Cytotoxicity Effects of Bentonite, Zeolite and Sepiolite Clay Minerals on Peripheral Blood Mononuclear Cells (PBMCs)

Fariba Fariba Nemati Shamsabad[1], Mohammad Hassan Salehi[2], Jalaleddin Sham[3], Tooba Ghazanfari[4]

1. Immunoregultion Research Center, Shahed University, Tehran, IRAN
2. Soil Science Department, College of Agriculture, Shahrekord University, Shahrekord, IRAN
3. Department of Oncology and Hematology, Shahed University, Tehran, IRAN
4. Immunoregultion Research Center, Shahed University, Tehran, IRAN

Background: Clays and clay minerals have great potential for exert positive effects on human health and implementation in medical applications. Clay minerals are industrial minerals used in various medical applications, such as drug delivery. Considering the abundance of clay resources in Iran, we decided to investigate the role of natural clays on peripheral blood mononuclear cells (PBMCs), which are the effective cells of the immune system and provide the health of the body in health and disease. Investigating the cytotoxicity of these minerals on PBMCs helps to understand their performance in medicine and the treatment of patients.

Methods: The studied clays including bentonite, zeolite, and sepiolite were extracted from Iran mines. Physical and chemical characterizations of clays examined by X-ray fluorescence (XRF) and X-ray diffraction, respectively. Peripheral Blood Mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation. The amount of 200000 PBMC cells were exposed to different concentrations of clays (1-1000 µg/ml) for 48 h in 96-Well Cell Culture Plates. Cell cytotoxicity response was determined according to 3- [4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay.

Results: The results showed that bentonite inhibited cell proliferation after 48 h incubation at the concentration above 0.05 mg/ml whereas zeolite in 10 and 5mg/ml. Sepiolite had any cytotoxic effect at all of concentrations. Results also indicated that the toxicity of these substances and their inhibition of cell growth strongly depend on the dose, type and characteristics of the studied clay minerals. The behavior of clays can be related to the particle size, shape and concentration. One of the factors in the occurrence of this behavior can be related to the flocculation of clay colloids at increasing concentrations and in the high salt concentrations of cell culture media.

Conclusion: Our findings showed that the cytotoxicity of the investigated clays is less than those observed in the literature review. This suggests that the studied clays with useful properties can be used in medicine, taking into account the size, type and concentration of clays. Our results could provide a new perspective on the safety of using these inexpensive and naturally and economical available clays in medical and industrial applications.

Keywords: PBMCs, Cytotoxicity, MTT, Clay minerals

Decrease in the Inflammatory Cytokines of LPS-Stimulated PBMCs of Patients with Atherosclerosis by a TLR-4 Antagonist in the Co-culture with HUVECs

Atefe Ghamar Talepoor[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Toll-like receptors (TLRs) are among the players of inflammation during atherosclerosis.

Methods: We assessed the effects of Eritoran, a TLR-4 antagonist, on lipopolysaccharide (LPS)-induced cytokines production by Peripheral Blood Mononuclear Cells (PBMCs) of patients with high-stenosis (HS) (n = 6) and healthy controls (HCs) (n = 6) co- cultured with Human Umbilical Vein Endothelial Cells (HUVECs).

Results: LPS stimulation significantly increased the levels of IL-6 (p=0.007 and p=0.005), TNF-α (p=0.006 and p=0.005), IL-2 (p=0.007 andp =0.002), IFN-γ (p=0.006 and p=0.003), IL-17A (p=0.004 and p=0.003), IL-17F (p=0.005 andp =0.003), IL-5 (p=0.007 and p= 0.005), IL-13 (p= 0.006 and p= 0.005), IL-9 (p= 0.005 and p=0.005) and IL-21 (p= 0.007 and p= 0.005) in HUVECs co-cultured with HC and HS PBMCs as compared with un-stimulated co-culture condition, respectively.

Conclusion: Our results demonstrate that attenuating effect of Eritoran on the inflammatory responses to LPS is higher in PBMCs of patients with high stenosis, suggesting its potential role in ameliorating inflammatory conditions in atherosclerosis.

Keywords: Lipopolysaccharide, Eritoran tetrasodium, Toll like receptor, Antagonist, Cytokine, Atherosclerosis

Effect of HM-Exos on the Migration and Inflammatory Response of LPS-Exposed Dental Pulp Stem Cells

Ehsaneh Azaryan[1], Samira Karbasi[1], Mansoore Saharkhiz[1], Mohammad Yahya Hanafi- Bojd[1], Asghar Zarban[1], Fariba Emadian Razavi[1], Mohsen Naseri[1]

1. Birjand University of Medical Sciences, Birjand, Iran.

Background: One of the prevalent dental conditions known as pulpitis is an opportunistic infection caused by oral bacteria, which causes tooth pulp inflammation. The purpose of this study was to investigate the effects of human milk exosomes (HM-Exos) on the viability, migration, and inflammatory responses of lipopolysaccharide (LPS)-exposed human dental pulp stem cells (HDPSCs) in vitro.

Methods: HM-Exos were isolated and dynamic light scattering (DLS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were used to analyze their physical properties (size and shape). To construct an in vitro inflammation model, HDPSCs were exposed to LPS. The MTT test and migration assay were used to investigate the effect of HM-Exos on cell proliferation and migration. The quantitative polymerase chain reaction (qPCR) was used to assess the expression of inflammatory genes in HDPSCs.

Results: DLS measurement revealed that HM-Exos were 116.8±3.6 nm in diameter. The SEM and TEM images showed spherical shapes with diameters of 97.2±34.6 nm. According to the cell viability assay results, the nontoxic concentration of HM-Exos was chosen for the subsequent investigations. The migration assay results showed that HM-Exos improved the potential of LPS-exposed HDPSCs to migrate. The qPCR results indicated that HM-Exos significantly reduced the expression of inflammatory cytokines such as TNF-α ($p<0.05$), IL-1β ($p<0.01$), and IL-6 ($p<0.01$) in HDPSCs after LPS stimulation.

Conclusions: HM-Exos increased LPS-exposed HDPSCs migration and proliferation and reduced gene expression of inflammatory cytokines. They may be a viable candidate for pulpitis therapy.

Keywords: Dental pulp stem cells, Exosomes, Inflammation, Lipopolysaccharide

Effect of Honey as an Immunomodulator on Human Dermal Fibroblast: A Comparative in vitro Study

Ehsaneh Azaryan[1], Samira Karbasi[2], Asghar Zarban[3], Mohsen Naseri[4]

1. Birjand University of Medical Sciences, Birjand, Iran.

Background: Jujube is a native and economically important Iranian herb that has primarily been cultivated in South Khorasan. Jujube honey (JH) has a higher content of antioxidants compared to other honeys, which have been related to a lower risk of heart disease, and protection against oxidative stress of free radicals. The objective of this study was to compare the effect of JH and commercial honey (CH) on viability and relative expression of immunoregulatory genes in human dermal fibroblast (HDF).

Methods: A honey health assessment was done based on honey's total phenolic content, antioxidant capacity, and diastase activity. In this way, the total phenolic content, antioxidant capacity, and diastase activity of JH and CH were measured. Cultured HDFs were exposed to various doses of JH and CH, and the viability of the exposed HDFs was assessed. The quantitative polymerase chain reaction (q-PCR) was used to evaluate the effect of honeys on immunomodulatory genes expression in HDFs.

Results: The total phenolic content of JH was 606.4 ±0.11 µg Gallic acid equivalent per mg, while CH honey had a value of 112.1 ±0.095. Total antioxidant activity of JH was compared to that of CH. In JH and CH, it was 203.58±10.5µM/L and 4.66±10.5 µM/L, respectively. A nontoxic concentration of JH and CH was determined for further experiments. Furthermore, q-PCR findings showed that JH dramatically boosted the expression of immunomodulatory genes including HLAG5, IL-6 and VEGF-A (p<0.05) when compared to the CH group.

Conclusions: The honey samples efficiently affected the proliferation and viability of HDFs. Moreover, JH can enhance the expression of immunomodulatory genes in HDFs more than CH.

Keywords: Honey, Human dermal fibroblast, Immunomodulatory

Effect of Hydroalcoholic Elaeagnus Angustifolia Extract on Immunomodulatory Genes and Wound Healing in Human Fibroblast Cells

Ehsaneh Azaryan[1], Sepideh Sarfi[2], Mohsen Naseri[3]

1. Cellular and Molecular Research Center, Department of Molecular Medicine, Birjand University of Medical Sciences, Birjand, Iran
2. Department of Immunology School of Medicine Birjand University of Medical Sciences
3. Cellular and Molecular Research Center, Department of Molecular Medicine, Birjand University of Medical Sciences, Birjand, Iran

Background: The objective of this study was to investigate the effect of hydroalcoholic Elaeagnus Angustifolia (EA) extract on viability, migration and relative expression of angiogenesis and immunomodulatory genes in human dermal fibroblast (HDF).

Methods: Cultured HDFs were exposed to various doses of EA, and the viability of the exposed HDFs was assessed. The rate of cell wound closure was then investigated. The quantitative polymerase chain reaction (q-PCR) was used to evaluate the effect of EA on angiogenesis (VEGF) and immunomodulatory genes (HLA-G5, IL-6) expression in HDFs.

Results: According to the result of the MTT assay, EA had no cytotoxic effect on cell viability for 24h. The migration test findings demonstrated that EA increased the ability of HDFs to move as compared to the control group ($p<0.05$). q-PCR findings showed that EA dramatically boosted the expression of angiogenesis and immunomodulatory genes ($p<0.01$).

Conclusions: The EA efficiently increased wound healing and the relative expression of angiogenesis and immunomodulatory genes in HDFs

Keywords: Elaeagnus Angustifolia, Human dermal fibroblast, Immunomodulatory, Angiogenesis

Effect of PCL/nHA-EA Nanocomposite on the Inflammatory Response of Lipopolysaccharide-Exposed Dental Pulp Stem Cells

Ehsaneh Azaryan[1], Mohammad Yahya Hanafi-Bojd[1], Esmat Alemzadeh[1], Fariba Emadian Razavi[1], Mohsen Naseri[1]

1. Birjand University of Medical Sciences, Birjand, Iran.

Background: Pulpitis is a common inflammation of tooth pulp tissue, and oral microbes are implicated in this opportunistic infection. Plants are still discovering new uses in contemporary days, and combining biocompatible scaffolds with various plant extracts can beneficially enhance scaffold bioactivity. In this study, nanohydroxyapatite (nHA) was synthesized using Elaeagnus angustifolia (EA) extract and added onto polycaprolactone (PCL) nanofibers (PCL/nHA-EA) to decrease of inflammatory cytokines in lipopolysaccharide (LPS) exposed human dental pulp stem cells (DPSCs) in vitro condition.

Methods: Modified nHA via EA extract (nHA-EA) synthesized with the sol-gel technique. Then nHA and nHA-EA incorporated into PCL and nanocomposites were prepared through the electrospinning method. The chemical properties and size of the nanofibers were assessed using Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM). To construct an in vitro inflammation model, DPSCs were exposed to LPS. MTT assay was used to determine cell viability and the quantitative polymerase chain reaction (qPCR) was used to assess the expression of inflammatory genes in DPSCs.

Results: According to the result of the MTT assay, PCL/nHA-EA nanofibers had no cytotoxic effect on DPSCs for 24h. The qPCR results indicated that LPS (1µg/ml) induced an inflammatory condition in DPSCs by increasing the expression of TNF-α, IL-1β and, IL-6 (p<0.05) and PCL/nHA-EA significantly reduced the expression of inflammatory cytokines after LPS stimulation (p<0.05).

Conclusion: PCL/nHA-EA nanocomposites had a noticeable effect on the reduced gene expression of inflammatory cytokines and may be a useful candidate for pulpitis therapy.

Keywords: Dental pulp stem cells, Elaeagnus angustifolia, Hydroxyapatite, Inflammation, Lipopolysaccharide

Evaluation of Monocyte Response due to Implant of a Controlled Released Drug Delivery System of Chitosan Hydrogel Loaded with Selenium Nanoparticle in Rats with Experimental Spinal Cord Injury

Maryam Nafar sefiddashti[1]

1. Shahrekord University, Shahrekord, Iran.

Background: Spinal cord injury (SCI) is one of the major injuries of the central nervous system and a devastating neurological disorder that can affect any individual at a given instance. The purpose of this study was to evaluate Monocyte changes following implantation of a controlled drug delivery system of chitosan hydrogel loaded with selenium nanoparticles in rats with SCI.

Methods: For this purpose, 60 adult female rats with experimental thoracic spinal cord compression (done by aneurysm clip) were divided into three equal groups: 1. control group (did not receive any medication), 2. Chitosan group (received chitosan hydrogels), and 3. Nano selenium group (received chitosan hydrogels containing selenium nanoparticles). Monocyte cell count was measured on days 3, 7, 21, and 28 after induction of spinal cord injury.

Results: In this study, no significant changes were observed in the average values of this parameter in certain times and groups.

Conclusion: Monocytes adhere to the endothelium and differentiate into macrophages. This takes place through L-selectin. According to previous studies, Oral administration of selenium prohibits monocytes adhesion to endothelium and as a result, their number will increase. In this study, it seems that due to the difference in the method of selenium administration (implantation instead of oral administration) and differentiation of monocytes into macrophages caused the decrease in their number.

Keywords: Spinal cord injury, Selenium nanoparticles, Chitosan hydrogel, Monocyte

Evaluation of RAW 264.7 Macrophage Cytokine Expressions Affected by Opium and Detoxification Drugs

Iman Farahani[1], Amir Mohammad Saeedifar[2], Ali Ghazavi[3], Ghasem Mosayebi[2], Parnian Navabi3, Ali Ganji[4]

1. Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran.
2. Department of Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.
3. Traditional and Complementary Medicine Research Center (TCMRC), Department of Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.
6. Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran

Background: Opium could have some detrimental effects on the immune system functions, like the inhibitory effect on macrophages. As line of treatment, detoxification drugs prevalently used for drug withdrawal programs. In the present study the effects of opium and its mentioned detoxification drugs on cytokines secreted by RAW 264.7 macrophages were investigated.

Methods: In the present study the effects of opium and its mentioned detoxification drugs including Buprenorphine (BUP), Naltrexone (NTX), and Methadone (MTD) on cytokines secreted by RAW 264.7 macrophages were investigated by Real-time PCR in triplicate. LPS and DMSO induced macrophages were used as the M1 model (positive control) and M0 model (negative control), respectively.

Results: Firstly, IC50 of each mentioned drugs calculated by the MTT assay were 150 µmol/L for OPM, as well as 3 µmol/L (BUP), 16 µmol/L (NTX) and 60 µmol/L (MTD).The ratios of inflammatory (IFN-γ, TNF-α, IL-6) to non-inflammatory cytokines (IL-10, TGF-β) of macrophage cells exposed to OPM and its detoxification drugs were measured by Real-time PCR. It was found that the most significant increase in the ratio of IFN-γ/IL-10 was found in OPM+MTD (p<0.001) and OPM+BUP (p<0.05) compared to M0 macrophages and the highest rise compared to the negative control was seen in OPM+MTD (p<0.001), OPM+BUP (p<0.01), OPM+NTX (p<0.05) groups is about INF-γ/TGF-β ratio. The highest increase compared to the M0 model was observed in OPM+BUP (p<0.001) and OPM+MTD (p<0.01) in terms of both TNF-α/ IL10 and TNF-α/ TGF-β. Furthermore, OPM+BUP (P<0.001) and OPM+MTD (P<0.05) showed the greatest increase in the ratio of IL-6/IL10 and OPM+BUP (p<0.001) also had an upward trend in the ratio of IL-6/TGF-β in a significant manner.

Conclusion: As a result, in relation to the increment in the ratio of inflammatory to non-inflammatory cytokines, the most significant effect was observed in OPM+BUP and OPM+MTD groups.

Keywords: Opium, Macrophage, Inflammation, Detoxification drugs

Exploring the PCGEM1/miR-433-3P/ITGA4 and PVT1/miR-183-5p/ITGB1 Axis in Patients with Rheumatoid Arthritis during Treatment with Conventional DMARDs and Methylprednisolone

Elham Faryadi[1], Alireza Rezaiemanesh[2], Nastaran Mohammadi[3], Seyed Askar Roghani[4], Shirin Assar[5]

1. Student Research Committee, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
3. Student Research Committee, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
4. Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran
5. Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

Background: Rheumatoid arthritis (RA) is a multifactorial autoinflammatory disease that predominantly affects the joints. Due to the use of conventional therapy, including conventional disease-modifying antirheumatic drugs (cMARDs) and Methylprednisolone (mPRED), there have been notable advancements in managing RA. This study aims to investigate the expression of ncRNAs regulatory axes and their possible downstream genes integrin alpha 4 and beta 1 (ITGA4 and ITGB1) in patients with RA after treatment.

Methods: 31 newly diagnosed RA patients and 27 healthy controls (HCs) were enrolled. For six months, patients were treated with cDMARDs and mPRED. Blood samples were collected from HSc and patients (before and after treatment). Following RNA extraction, the RT-qPCR method was applied to determine the expression level of the investigated genes.

Results: After exploring two axes, a significant decrease in miR433-3p and miR-183-5p (p= 0.034 and P=0.01, respectively) and increased expression of ITGA4 and ITGB1 (p= 0.003 and p=0.022, respectively) was observed in newly diagnosed patients compared to HCs. After treatment, the expression level of PCGEM1, PVT1, ITGA4, and ITGB1 significantly decreased (p= 0.003, p= 0.004, p= 0.002, and p= 0.0001, respectively) and miR-183-5p remarkedly increased (P= 0.022).

Conclusion: Our data demonstrated that the cDMARDs + mPRED treatment could reduce ITGA4 and ITGB1 in RA patients. Also, PVT1/miR-183-5p/ITGB1 axis may play a role in this therapeutic pathway in rheumatoid arthritis.

Keywords: Rheumatoid arthritis, DMARDs, lncRNA, miRNA, Integrin

Expression of Immunomodulatory Biomarkers in Human Dental Pulp Mesenchymal Stem Cells Treated with Curcumin

Mansoore Saharkhiz[1], Malaksima Ayadilord[2], Mohsen Naseri[3], Fariba Emadian Razavi[4]

1. Department of Immunology, Birjand University of Medical Sciences, Birjand, Iran
2. Department of Immunology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.
4. Department of Prosthodontics, Birjand University of Medical Sciences, Birjand, Iran.

Background: Human Dental pulp mesenchymal stem cells (hDP-MSCs) have tissue regeneration and immunomodulatory properties. Therefore, they are ideal candidates for regenerating damaged tissues and treating inflammation-related diseases. However, methods (such as genetic variation) to improve the immunomodulatory and regenerative efficiency of MSCs still need to be developed. Curcumin (CUR) is known for its broad applications in regenerative medicine and the treatment of inflammatory disorders. This study was conducted to investigate the effects and underlying mechanisms of CUR on the immunomodulatory function of hDP-MSCs and whether treating these cells with CUR can improve therapeutic efficacy.

Methods: hDP-MSCs were isolated from dental pulp and characterized according to predetermined criteria including flow cytometric analysis and multilineage differentiation potential. Cell viability rate was investigated by MTT assay. Real-time quantitative (RT-PCR) was applied to investigate the expression of immunomodulatory genes after cell treatment with CUR.

Results: The results showed that VEGF-A and STAT3 markers were up-regulated while HLA-G5 and VCAM-1 markers were down-regulated by CUR treatment in hDP-MSCs ($p< 0.001$). Besides, this research indicated that there were no significant changes in the expression of RelA after 48 h ($p= 0.33$).

Conclusion: Findings demonstrate that CUR can enhance the immunomodulatory effects of hDP-MSCs and improve their therapeutic efficacy. These findings can give an understanding of the mechanism for improving immunomodulatory activity in hDP-MSCs by curcumin.

Keywords: Dental pulp stem cell, Immunoregulatory, Immunomodulatory, Curcumin

Glatiramer Acetate Attenuates Renal Ischemia Reperfusion Injury in Rat Model

Sadaf Nezamoleslami[1], Mohammad Sheibani[2], Ahmad Reza Dehpour[1], Hamed Shafaroodi[1]

1. Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran.
2. Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Chronic renal failure can ultimately lead to kidney transplantation. Renal transplantation is associated with ischemia-reperfusion injury (I/R). The subsequent processes of kidney I/R can lead to irreversible damages to the kidney tissue. Glatiramer acetate is an immunomodulatory drug for the treatment of multiple sclerosis (MS) and the anti-inflammatory effects of this drug have already been proven in some inflammatory models. The purpose of this study was to evaluate the protective effects of Glatiramer on reducing the damages arising from kidney ischemia-reperfusion.

Methods: In this study, 35 Wistar rats were used which divided into 5 groups: sham, control (I/R), I/R+Glatiramer 0.5 mg/kg, I/R+Glatiramer 1 mg/kg, I/R+Glatiramer 2 mg/kg. Renal arteries were clamped bilaterally for 45 min, then the clamps were removed and the reperfusion process continued to 24 h. In the following, serum and kidneys were separated for analysis.

Results: In the control group, serum levels of LDH, inflammatory factor TNF-α and renal functional markers such as BUN and Creatinine were remarkably increased, but in the treatment groups, especially in Glatiramer 2 mg/kg received group, a significant decrease in these factors was observed. Tissue concentration of MDA was reduced following Glatiramer treatment. Besides, Glatiramer attenuated the increased kidney level of NF-κB protein using immunohistochemical assay. NFκB migration to the nucleolus increases inflammatory cytokines production. The anti-inflammatory factor, IL-10, in serum was significantly increased in the treatment group of Glatiramer 2 mg/kg. Furthermore, Glatiramer decreased renal tissue injury score according to the histopathological study.

Conclusion: These results demonstrate that Glatiramer may play protective effects in kidney ischemia-reperfusion injury by reducing inflammatory and oxidative damages.

Keywords: Glatiramer, Ischemia/reperfusion, Kidney, Inflammation

Glatiramer Acetate Treatment Inhibits Inflammatory Responses and Improves Survival in A Mice Model of Cecal Ligation and Puncture-Induced Sepsis

Elahe Maleki[1], Mohammad Sheibani[2], Sadaf Nezamoleslami[2], Ahmad Rerza Dehpour[2], Nasrin Takzaree[3], Hamed Shafaroodi[2]

1. Faculty of Pharmacy and Pharmaceutical Science, Islamic Azad University of Medical Science, Tehran, Iran
2. Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
3. Department of Anatomy & Histology, School of Medicine, Tehran University of Medical Science, Tehran, Iran

Background: Sepsis is a clinical crisis which has been considered as one of the important causes of mortality across the world. We hypothesized that modulation of hyper-inflammatory phase of sepsis pathophysiology can lead to protective effects on survival outcome. Glatiramer acetate (GA) is a neuroprotective drug commonly used in multiple sclerosis (MS). GA is characterized by immune activity via regulation of innate and adaptive immunity. This study was designed to evaluate the acute treatment with GA on initial inflammatory response-induced mortality in septic mice.

Methods: Cecal ligation and puncture (CLP) model was operated on male mice as a model of polymicrobial sepsis. GA was administrated intraperitoneally after the sepsis induction at doses of 0.5, 1, and 2 mg/kg in three treatment groups. To investigate the effect of GA on short-term survival, septic mice were observed during 72 h after CLP. Serum levels of TNF-α, IL-1β, and IL-6 as pro-inflammatory cytokines and also IL-10 as a critical anti-inflammatory cytokine were analyzed. To consider sepsis-induced acute kidney injury, renal functional biomarkers and histopathological changes was assessed.

Results: GA treatment significantly improved survival rate at doses of 1, and 2 mg/kg. Survival improvement was accompanied by remarkable reduction in the pro-inflammatory cytokines and enhanced production of IL-10. GA showed to have protective effects on renal function as well.

Conclusion: Immunomodulatory and anti-inflammatory properties of GA resulted in increase in survival rate and decrease in inflammatory markers in mice model of cecal ligation and puncture–induced sepsis.

Keywords: Glatiramer acetate, Inflammation, Mice, Sepsis, Survival

IFN-β Modulated Immune System by Effecting Expression of IL-27, IL-17, VLA4, MCAM and CD80 in Experimental Autoimmune Encephalomyelitis Induced Mice

Rasul Karimi[1], Abbas Ali Amini[2]

1. Cellular and Molecular Research, Research institute for health devalopment, Kurdistan University of Medical Sciences, Sanandaj_iran
2. Department of Immunology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj_iran

Background: Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of central nervous system (CNS). Although the cause of the disease is still unknown, but due to demyelination and subsequent loss of axons, it results in Physical disability. Interferon beta is one of the most widely used treatments for MS. This drug reduces the number of attacks and slows the progression of disability. In fact, interferon beta reduces the immune response in patients with MS and increases regulatory T cells. In this study, the effect of interferon beta on IL-17, CD80, MCAM (CD146), VLA4 and IL-27 investigated.

Methods: 6-8 weeks old C57BL/6 mice were used and divided into three groups: EAE induced group that treated with interferon beta; EAE induced group which treated with saline buffer phosphate (PBS) and the third group of healthy control mice without receiving PBS or interferon beta. Two treated groups received IFN-β (10,000 IU) and PBS every other day. Mice killed twenty days after starting treatment and their brains were isolated. All brain tissues used to extract mRNA. Following cDNA construction, the expression of IL-17, CD80, MCAM (CD146), VLA4 and IL-27 genes investigated.

Results: In mice with EAE, the expression of all studied factors increased significantly compared to the healthy group. Interferon-beta treatment significantly reduced the expression of IL-17, CD80, MCAM and VLA4 in IFN-β treated mice compared to untreated EAE induced mice, nonetheless IL-27 expression was significantly increased in two EAE induced groups compared to the healthy control group, there was no significant difference between the two groups of IFN-β and PBS treated mice.

Conclusion: IFN-β decreased the severity of the disease and reduced CNS damage by decreasing the inflammatory and migratory factors of T lymphocytes. Treatment with this agent modulated the immune system and created conditions resembling healthy mice

Keywords: EAE ،Multiple sclerosis ،βIFN ،IL-17 ،CD80 ،MCAM ،VLA4

Immune-Related Adverse Events (IRAEs) in Ankylosing Spondylitis (AS) Patients Treated with Interleukin (IL)-17 Inhibitors: A Systematic Review and Meta-Analysis

Hossein Azadeh[1], Reza Alizadeh-Navaei[2], Alireza Rezaiemanesh[3], Misagh Rajabinejad[4]

1. Department of Internal Medicine, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Gastrointestinal Cancer Research Center, Non-Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran
3. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
4. Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease characterized by immune system dysregulation and inflammation in the joints. Interleukin (IL)-17 inhibitors are new biological drugs used to treat AS. In this study, we aimed to assess the risk of immune system-related adverse events (irAEs) due to targeting IL-17 or IL-17R.

Methods: The CENTRAL, PubMed, Scopus, Google Scholar, Clinical Trials Registry, and ICTRP were searched for Randomized clinical trials (RCTs) and non-RCTs until February 2021. The risk of irAEs in patients treated with IL-17 inhibitors compared to the placebo or a drug-free control was evaluated. In studies that reported AEs of the IL-17 inhibitors at several different time points, we compared the number of cases/100 patient-year in which irAEs were reported. Subgroup analyses were also performed based on the dose and type of drugs.

Results: Thirteen studies of 1848 AS patients treated by IL-17 inhibitors (secukinumab, ixekizumab, bimekizumab, and netakimab) and 764 participants who received a placebo were included. The risk of some AEs related to immune function in patients under IL-17 inhibitors treatment was significantly higher than that of the placebo group, including infection and infestation (Risk difference RD = 0.09, $p = 0.02$), nasopharyngitis (RD = 0.04, $p < 0.001$), opportunistic infections (RD = 0.01, $P = 0.04$), and neutropenia (RD = 0.04, $p = 0.03$). Besides, the results of the Cochran Q test showed that there were significant differences between the occurrence of some AEs over time, including infection and infestations ($p < 0.001$, RCTs), upper respiratory tract infections ($p < 0.001$, non-RCTs), urinary tract infections ($p < 0.001$, non-RCTs), and diarrhea ($p < 0.01$, RCTs).

Conclusion: The most common immune system-related AEs in patients treated with IL-17 inhibitors are mucosal and opportunistic infections.

Keywords: Ankylosing spondylitis (AS), Interleukin (IL)-17 inhibitors, Randomized controlled trial (RCT), Immune-related adverse events (irAEs).

Immunomodulatory Effects of Engineered hTERT-MSCs Overexpressing IDO1 Gene against Xenogeneic Rat Peripheral Blood Mononuclear Cells

Saeedeh Pourhamedi[1], Maryam Moghaddam Matin[1], Azadeh Haghighitalab[1], Ahmad Reza Bahrami[1*]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Mesenchymal stem cells (MSCs) exhibit amenable immunoregulatory properties mainly through their paracrine effects. Some of the factors involved in this process include indoleamine 2,3-dioxygenase (IDO), prostaglandin-E2 (PGE2), and nitric oxide (NO). IDO1 is the rate limiting enzyme in tryptophan catabolic pathway. Herein, we investigated the immunosuppressive properties of the engineered MSCs (hTERT-MSCs-IDO1) on rat peripheral blood mononuclear cells (rPBMCs).

Methods: Rat PBMCs were isolated from freshly collected rat blood samples. Cell isolation was carried out on Lymphodex (Inno-Train). Lymphocyte inhibition assays were performed following co-culture experiments between untreated-hTERT-MSCs, interferon gamma treated cells (250 U/ml), hTERT-MSCs-GFP, and hTERT-MSCs-IDO1 (MSCs to PBMCs ratio of 1:3). hTERT-MSCs-GFP and hTERT-MSCs-IDO1 represent hTERT-MSCs transduced with lentiviral vectors containing control backbone and *IDO1* gene, respectively. Following 3 and 5 days of co-culture experiments, cell viabilities of suspension cells were calculated using MTT assay.

Results: Functional analysis revealed that the hTERT-MSCs-IDO1 decreased the proliferation of xenogeneic rat PBMCs versus hTERT-MSCs-GFP (93.82% and 70.62% following 3 and 5 days of co-culture experiments, respectively). The highest level of inhibition was observed for IDO1 engineered cells as compared to other investigated groups. These differences were statistically significant on day 5 of co-culture.

Conclusion: Although, these results need more complementary studies, they confirm the immune-modulatory effects of hTERT-MSCs-IDO1 against rat PBMCs, mostly enriched for lymphocytes during isolation steps.

Keywords: hTERT-MSCs, Indoleamine 2, 3-dioxygenase (IDO1), Lymphocyte inhibition assay, Rat peripheral blood mononuclear cells.

Immunomodulatory Effects of Hydrogels in Treating Chronic Wound Healing: A Systematic Review

Pouria Ahmadi Simab[1], Mahdi Soroushianfar[2], Mahsa Khiyabani[2], Soheil Sadr[2], Hossein Kazemi Mehrjerdi[2]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
2. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: The incidence of chronic wounds in developed countries ranges from 1–2%. In order to improve patient's quality of life and maintain their physical and mental well-being, chronic wounds need to be well treated. Chronic wounds can be treated with debridement, ultrasound, hyperbaric oxygen therapy; negative pressure wound therapy, electromagnetic therapy, hydrogel dressings, and skin grafts. The tunable functional properties of hydrogel dressings, such as biodegradability, adhesiveness, antimicrobial, anti-inflammatory, and pre-angiogenic bioactivities, could be a promising option for chronic wound healing. This review aims to summarize the types of chronic wounds, phases of the healing process, and key approaches to treatment.

Methods: Eleven databases (Embase, Scopus, Google Scholar, PubMed, Cochrane Library, Magiran, UpToDate, SID, Medline, Elsevier, and Lilacs) were searched for published articles on Immunomodulatory effects of hydrogels in treating chronic wound healing from January 2002 to July 2022. Seventeen affiliated articles with complete abstracts were included in this study. All data were extracted from interrelated papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Hydrogel-based dressings can easily be modified compared to other chronic wound treatment strategies. Using hydrogel dressings can speed wound contraction and healing by loading cells, antiviral, antibacterial, and antifungal agents, biomolecules, and growth factors due to their flexible, tunable properties. Hydrogel dressings can be developed depending on the size, severity, and location of the wound. Because these dressings induce in situ and cytocompatible chemical crosslinking, they can also be easily applied to irregular or deep wounds.

Conclusion: Several advantages can be derived from hydrogel-based dressings, such as their ability to bind and adhere to the surface, their vascularization ability, antimicrobial properties, antioxidant activities, and anti-inflammatory properties.

Keywords: Antioxidants, Chronic wounds, Hydrogels, Wound healing

Immunomodulatory Effects of the Induced Pluripotent Stem Cells through Expressing IGF-related Factors and IL-10 in vitro

Paria Bayati[1], Marjan Taherian[1], Hadi Poormoghim[2], Nazanin Mojtabavi[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Rheumatology department, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran

Background: Among the novel approaches for treating some hard-to-cure diseases such as rheumatologic disorders are Induced Pluripotent Stem Cells (IPSCs). Aside from their regenerative capacities, some studies have shown the potential of these cells in the modulation of inflammatory responses. But their mechanism of action is not understood so far. Therefore, we aimed to investigate the expression of the genes associated with the IGF pathway as well as IL-10 and TGF-β, which are known to exert immunomodulatory effects.

Methods: C57/Bl6 mice were used for obtaining mouse embryonic fibroblasts (MEFs), then the IPScs were induced using lentiviral vectors expressing the pluripotency genes (OCT4, SOX2, KLF1, and c-MYC). Cells were cultured for 72 hours in DMEM high glucose plus leukemia inhibitory factor (LIF); evaluating the gene expression was conducted using specific primers for IGF1, IGF2, IGFBP3, IGFBP4, IRS1, IL-10, and TGF-β genes, as well as SYBR green qPCR master mix. The data have been analyzed using the 2-ΔΔCT method and were compared by employing t-test; the results were plotted using GraphPad PRISM software. MEFs were utilized as controls.

Results: Gene expression analyses revealed that IGF-1, IGF-BP3, IGF-BP4, and IL-10 were significantly upregulated ($p \leq 0.05$), while IGF-2 and TGF-b genes were significantly downregulated in the lysates from IPSCs compared with the control MEFs. The IRS gene expression was not altered significantly.

Conclusion: IPSCs are capable of modulating inflammatory responses through the expression of various anti-inflammatory factors such as IGFBPs as well as IL-10. This finding reveals a new aspect of the therapeutic impact of the IPSCs which could be translated into further in vivo studies.

Keywords: Immunomodulation, Induced pluripotent stem cells, IGF-1, IGF-2, IGFBP3, IGFBP4, IPSCs

Investigating the Effects of Lithium Chloride on Immunomodulatory Properties of Bone Marrow-Derived Mesenchymal Stem Cells

Halimeh Hassanzadeh[1], Ahmad Reza Bahrami[2], Hamid Reza Bidkhori[3], Mahboubeh Kazemi Noughabi[1], Maryam M. Matin[4]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran. Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Stem Cells and Regenerative Medicine Research Group, Academic Center for Education, Culture and Research (ACECR)-Khorasan Razavi, Mashhad, Iran
4. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran. Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Lithium chloride (LiCl) has been linked to an increase in bone mass when used as a treatment for mental disorders. Bone healing that is delayed due to acute or prolonged inflammation is a serious issue. In the early stages of the healing process, some chemokines and cytokines are released such as IL-6, causing an increase in immune cell migration and pro- inflammatory behavior in the injured area. To be able to proceed with the healing process however, it might be preferred if inflammation is attenuated. In terms of regenerative medicine, mesenchymal stem cells (MSCs) are an ideal cell source due to their differentiation capacity and also their immunomodulatory properties. We conducted this study in order to investigate whether incubation of bone marrow (BM)-derived MSCs with LiCl would affect the expression of several immunomodulatory genes.

Methods: BM-MSCs were obtained from the bone marrow aspirate of patients after obtaining their informed consent and the characteristics of the BM-MSCs were examined using flow cytometry as well as evaluating their differentiation potential using specific differentiation media. Afterwards, the samples were treated with LiCl (20 mM, for 24 h), and following RNA extraction and cDNA synthesis, the samples were evaluated for the expression of several immunomodulatory genes by real time PCR.

Results: Our experiment proved the identity of BM-MSCs through the overexpression of several cell surface markers, such as CD44, CD73, CD90, and CD105, which confirmed the identity of BM-MSCs. As a result of staining fat vacuoles and calcium deposits of differentiated cells, we were also able to confirm their multipotency. In BM-MSCs treated with LiCl, the expressions of immunomodulatory related genes including TDO-2, COX-2, and TSG-6 significantly increased (p <0.01) compared to the control group. A decrease (p<0.01) in the expression of IL-6, one of the pro-inflammatory cytokines, was also observed following the treatment of BM-MSCs with LiCl.

Conclusion: BM-MSCs treated with lithium chloride, were observed to exhibit profound up- and down-regulatory effects on the expression of immunomodulatory and pro-inflammatory genes, respectively, the phenomena associated with the anti-inflammatory phenotype of MSCs.

Keywords: Mesenchymal stem cells, Lithium chloride, Immunomodulatory, Bone healing

In-vitro Effects of Kaolin on IL-6 and TNF-a Cytokines Derived from PBMCs

Haniyeh Shahabi[1], Tooba Ghazanfari[2], Fariba Nemati Shamsabad[2], Davoud Jamali[1], Reza Sedaghat[4]

1. Department of Immunology, School of Medicine, Shahed University, Tehran, Iran
2. Immunoregulation Research Center, Shahed University, Tehran, Iran
3. Department of Immunology, School of Medicine, Shahed University, Tehran, Iran
4. Department of Anatomy and Pathology, School of Medicine, Shahed University, Tehran, Iran

Background: Kaolin as a clay-type mineral with Al2Si2O5(OH)4 composition has an outstanding potential to be used for biomedical purposes such as drug delivery, tissue engineering, and so on. Based on recent studies, this type of clay can influence groups of cytokine production, namely inflammatory ones. Since clay minerals can have different properties in different areas, assessing their biological effects in our country could be worthwhile. Herein, we evaluated the effect of Kaolin on the production of two important inflammatory cytokines: IL-6 and TNF-a.

Methods: Human PBMCs were obtained by the ficoll separation method. After adding 1 ml of RPMI to the cell plot, the cell count was measured. Subsequently, they were treated with four concentrations of Kaolin 0.005, 0.01, 0.5, and 0 mg/ml, and 200000 cells in each well were incubated for 24 hours (at 37˚Temperature,5% Co2, and 95% O2). Cell supernatants were separated for cytokine evaluation and Inflammatory cytokines, including IL-6 and TNF-a, were assessed through the ELISA method.

Results: Although we didn't see any significant changes in IL-6 production in four different concentrations, TNF-a production decreased in the highest-used concentration of Kaolin at 0.05 mg/ml level against the other three concentrations.

Conclusion: Based on our findings, Kaolin could decrease the TNF-a production at the highest concentration while we didn't see any significant changes in IL-6 cytokine level. As Kaolin minerals can play a beneficial role in medical practices such as acting as a nano drug carrier, their impact on immunological reactions like inflammatory cytokine production must be noted. This study provided useful insight into the impact of Kaolin on reducing TNF-a cytokine production. However, further studies must be done to broadly assess the impact of kaolin on the other inflammatory cytokine as well as other immunological pathways.

Keywords: IL-6, TNF-a, Peripheral blood mononuclear cells, Kaolin

M1 Macrophages-derived Exosomes Increased The Expression of M1 Phenotype-Related Markers

Mohammad Mahmoudi[1], Mahsa Taghavi-Farahabadi[2], Seyed Mahmoud Hashemi[3], Kazem Mousavizadeh[4], Nima Rezaei[2], Nazanin Mojtabavi[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Macrophages are the most abundant tumor-infiltrating immune cells. They are conventionally classified as M1 and M2 types. M2 type is the dominant phenotype of macrophages in the tumor microenvironment (TME). Since macrophages have plasticity, reprogramming them is one of the strategies in cancer immunotherapy. Exosomes are Nano-vesicles that carry the components of the cells that they originated. M1 macrophages contain the mRNAs, miRNAs, and proteins related to M1 phenotypes. So, we investigated the effects of exosomes derived from M1 macrophages on polarization and anti-tumor properties of macrophages.

Methods: In this study, the RAW264.7 murine macrophage/monocyte cell line was obtained and maintained in DMEM supplemented with 10% heat-inactivated FBS. When cells reached 80% confluence, the cell culture medium was replaced with a serum-free medium. Cells were stimulated with LPS and incubated for 24 hours. Then, the culture media of cells were collected and exosomes were extracted. The exosomes were characterized using flow cytometry, SEM, TEM and DLS. After treating of the RAW264.7 with exosomes, the expression of CD86 and CD206 was evaluated by flow cytometry.

Results: It was demonstrated that LPS induced macrophage polarization into M1 phenotype. Exosomes derived from M1 macrophages could increase the expression of CD86 and decrease the expression of CD206.

Conclusion: M1 Macrophages-derived exosomes can be used for reprogramming of macrophages as an important innate immune cell in TME.

Keywords: Macrophages, Exosomes, Tumor, Immunotherapy

M2 Macrophage Activation and Cutaneous Wound Healing: A Systematic Review

Pegah Moharrami Yeganeh[1], Abdolreza Esmaeilzadeh[1]

1. Zanjan University of Medical Sciences, Zanjan, Iran.

Background: Wounds and ulcers are major causes of morbidity and mortality. Macrophages and their potential roles in affecting the wound microenvironment and directing the healing process have become a source of interest in recent years. Macrophages are mainly "polarized" into two subtypes, M1 and M2, in the wound area. M1 and M2 macrophages are widely known as pro-inflammatory and anti-inflammatory macrophages, respectively. This study aims to investigate the role of M2 Macrophages in normal and pathological cutaneous wound healing.

Methods: This systematic review was performed to identify studies published in Science Direct, Scopus, PubMed databases, and Google Scholar search engine in the 2018 – July 2022 time interval using four keywords (M2 Macrophage, Immune Cell Therapy, Wound Healing, and Chronic Wound). Of the 114 identified articles based on our inclusion criteria, 38 articles were selected based on our exclusion criteria.

Results: M2 macrophages are known as "anti-inflammatory" macrophages marked by expression of CD64 and CD209. This macrophage phenotype is induced by T helper 2 cells and secretion of Macrophage colony-stimulating factor (M-CSF), Interleukin (IL)-4, IL-10, IL-13. These cells deliver anti-inflammatory and reparative functions through the production of cytokines, including monocyte chemo-attractant protein-1 (MCP)-1, IL-4, IP-10, macrophages inflammatory protein (MIP)-1β, and also endocytic and phagocytic activity. M2 macrophages contribute to wound closure, re-vascularization, fibroblast regeneration, and myofibroblast differentiation. Chronic wounds are characterized by increased inflammation and reduced m2 activity; thus, promoting m2 phenotype induction is proposed as an effective intervention in facilitating chronic wound repair.

Conclusion: A healthy balance between different phenotypes of macrophages is necessary for normal wound repair. Inducing and promoting the M2 macrophage phenotype activation can have beneficial effects in repairing chronic and non-healing cutaneous wounds such as diabetic ulcers.

Keywords: M2 Macrophage, Immune Cell Therapy, Wound Healing, Chronic Wound

Progranulin (PGRN) as a Critical Factor in the Immunopathogenesis of Cardiovascular Diseases

Ali Saeedi-Boroujeni[1], Daryush Purrahman[2], Ali Shojaeian[3], Łukasz A Poniatowski[4], Fatemeh Rafiee[5], Mohammad-Reza Mahmoudian-Sani[6]

1. Department of Microbiology, School of Medicine, Abadan University of Medical Sciences, Abadan, Iran
2. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
4. Department of Neurosurgery, Dietrich-Bonhoeffer-Klinikum, Neubrandenburg, Germany
5. Zanjan Metabolic Diseases Research Center, Zanjan University of Medical Science, Zanjan, Iran
6. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: progranulin (PGRN) serves as one of the main components of the regulation of inflammatory processes, which significantly contributes to the immunopathogenesis of such disorders. This review aims to summarize the role of PGRN in the immunopathogenesis of CVD, with an emphasis on its treatment.

Methods: The used search terms were Progranulin, PGRN, cardiovascular diseases inflammation, coronavirus, SARS-CoV-2 and NLRP3 inflammasome in Title/Keywords/Abstract. Each database was searched independently. The articles retrieved from both (Web of Science and PubMed) databases were analyzed once. Abstracts were reviewed based on predefined inclusion and exclusion criteria.

Results: The full-length PGRN can thus effectively reduce the calcification of valve interstitial cells, and the granulin precursor (GRN), among the degradation products of PGRN, can be beneficial. Moreover, it was observed that, PGRN protects the heart against ischemia-reperfusion injury. Above all, PGRN also provides protection in the initial phase following myocardial ischemia-reperfusion injury. The protective impact of PGRN on this may be associated with the early activation of the PI3K/Akt signaling pathway. PGRN also acts as a protective factor in hyperhomocysteinemia, probably by down-regulating the wingless-related integration site Wnt/β-catenin signaling pathway. Many studies have further demonstrated that SARS-CoV-2 (COVID-19) has dramatically increased the risks of CVDs due to inflammation, so PGRN has drawn much more attention among scholars. Lysosomes play a pivotal role in the inflammation process, and PGRN is one of the key regulators in their functioning, which contributes to the immunomodulatory mechanism in the pathogenesis of CVDs.

Conclusion: As a regulatory and mainly anti-inflammatory factor whose role has been shown in many and autoimmune diseases, PGRN can be thus considered as a missing link in the chain of the events of CVD immunopathogenesis. Investigation of PGRN actions can help find new prospects in the treatment of CVDs.

Keywords: Progranulin, Cardiovascular Diseases, Inflammation, PGRN

STAT3 Inhibition Induces Adipose-Derived Mesenchymal Stem Cells with Anti-Inflammatory Properties

Fatemeh Khammari Amir Salek Farrokhi[1]

1. Department of immunology, Faculty of Medical Sciences Semnan University, Semnan, Iran.

Background: Mesenchymal stem cells (MSCs) have naturally limited ability to modulate the innate and acquired immune system by inhibiting inflammatory responses and stimulating anti-inflammatory activities. Therefore, the aim of this study is to investigate the effects of STAT3 inhibition on immunomodulatory properties of MSCs.

Methods: Mesenchymal stem cells isolated from adipose tissue of BALB/c mice through collagenase enzyme method. The survival rate of MSCs was evaluated following treatment by S3I-201 (1, 10, 20, 60, 80, 100 µM) through MTT method. Phenotype characterization of MSCs was evaluated through differentiation to adipocyte, osteocyte, and flow cytometry analysis of surface markers (SAC-1, CD29, CD73, CD90, CD105, CD11b, CD34, and CD45). MSCs were treated with S3I-201 as a STAT3-inhibitor, and then we measured TNFα ،TGFβ ،IL10 concentration in MSCs supernatant by ELISA and, the expression of BAX, BCL-2, Caspase3, IL-10, IDO, TNF-α and TGF-β genes was evaluated by Real-time-PCR. The activity of IDO enzyme in MSCs supernatant was investigated by spectrophotometric method. The apoptosis rate of MSCs was studied by Annexin-PI staining as well as nitric oxide (NO) factor was measured using Griess solution. We evaluated the proliferation of splenocytes of BALB/c mice in the presence of MSCs-conditioned medium treated with S3I-201.

Results: Inhibition of STAT3 has not significant effects on survival and apoptosis rate of MSCs. Moreover, there was no significant alteration in the surface markers (SAC-1, CD29, CD73, CD90, CD105, CD11b, CD34, and CD45) of MSCs and their differentiation into adipocyte and osteocyte following treatment with S3I-201. The results showed increased expression of IL-10, IDO, and TGF-β genes accompanied with decreased level of TNFα gene that could increase immunomodulatory properties of MSCs. Data showed that TGF and IL-10 production increased and TNF-α decreased from MSCs following treatment with S3I201. STAT3 inhibition significantly increased production of NO and activity of IDO enzyme in the MSCs. The results showed that the conditioned medium derived from MSCs treated with S3I-201 inhibited the proliferation of the splenocytes of BALB/c mice.

Conclusion: Our data showed that S3I-201 increases the immunomodulatory properties of MSCs and we could investigate the therapeutic application of MSCs following STAT3 inhibition in various inflammatory and autoimmune diseases.

Keywords: Mesenchymal stem cells, STAT3, S3I-201, Immunomodulation

The Effect of Bentonite Clay on IL6 and TNF-α Secretion from Human PBMCs

Mahdiyeh Nateghi[1], Fariba Nemati Shamsabad[2], Davoud Jamali[1], Reza Sedaghat[3], Tooba Ghazanfari[2]

1. Immunoregulation Research Center, Shahed University, Tehran, Iran 2. Department of Immunology, School of Medicine, Shahed University, Tehran, Iran
2. Immunoregulation Research Center, Shahed University, Tehran, Iran
3. Department of Anatomy and Pathology, School of Medicine, Shahed University, Tehran, Iran

Background: Bentonite is one of the most applicable clays in the industry because of its properties such as adsorbability, ionic exchanger, high abundance, easy manipulation, and eco-friendly. Recently, it has attracted a lot of attention in the medical field as it acts like a potential agent in delaying drug release, targeting drug release, preventing or reducing side effects of drugs, namely chemotherapy drugs, and increasing the stability of drugs. Although its health benefits were reported in numerous studies, some in vitro studies indicated that bentonite could cause a high degree of cytotoxicity on different types of mammalian cells, promoting oxidative stress and cell membrane damage. The aim of the current study was to investigate the effect of bentonite on pro-inflammatory cytokine production from human PBMCs in-vitro.

Methods: We administrated bentonite with three different concentrations (0.01, 0.0005, and 0.001 mg/ml) for 24 h and 48 h in the PBMCs culture (200.000 cells/well) of a healthy donor. Then, the levels of two pro-inflammatory cytokines, IL-6 and TNF-α, were assessed in the culture supernatants by ELISA.

Results: The results showed that dose 0.001 of bentonite clay could significantly increase the production of IL6 in the PBMC culture supernatants compared to the negative control. Moreover, the production of TNFα in our culture supernatants exhibited an upward trend in all the doses but only the highest dose induced a significant increase.

Conclusion: Regarding the positive applications of bentonite, we should be cautious about its dose-dependent adverse effects. In addition, it is necessary to determine the adequate dosage of bentonite, which has the least side effects and could provide what we expect in industrial approaches, with an emphasis on cosmetic, pharmacological, and food products.

Keywords: Bentonite, IL6, TNFα, PBMC

The Effects of Dexamethasone-Loaded Exosomes on Lung Injury in CLP-Induced Sepsis Mice

Zahra Mirsanei[1], Fatemeh Ahangari[1], Sara Soufi[2], Fatemeh Jamshidi[3], Solaiman Ali Alhashmi[3], Saeed Vakilian[3], Sara Soudi[2], Seyed Mahmoud Hashemi[1]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. University of Tarbiat Modaress, Tehran, Iran.
3. University of Nizwa

Background: The regenerative effect of umbilical cord mesenchymal stem cells-derived exosomes in tissue healing, along with the anti-inflammatory effect of dexamethasone on inflammatory diseases such as COVID-19, promoted us to examine the efficacy of dexamethasone-loaded exosomes on lung damage caused by sepsis.

Methods: Cecal ligation and puncture (CLP) model was used to induce sepsis in mice. C57BL/6 mice injected with sham, CLP-induced sepsis, CLP-induced sepsis mice injected with exosomes, CLP-induced sepsis mice injected with dexamethasone, and CLP-induced sepsis mice injected with dexamethasone-loaded exosomes. The mice were sacrificed after 24 hours, tissue samples were collected for H&E staining, and BAL fluid was collected to examine protein and leukocyte changes.

Results: According to the results of a study conducted on sepsis mice, in comparison to the CLP group, the number of infiltrated immune cells, congestion, and alveolar wall thickness in lung tissue, and the total protein and leukocyte population in BAL fluid were significantly reduced in the dexamethasone-loaded exosomes treated group.

Conclusion: As a nanoparticle carrying very small amounts of dexamethasone compared to the administration of dexamethasone alone, exosomes loaded with dexamethasone can effectively reduce pulmonary inflammation in sepsis patients and reducing systemic dexamethasone side effects.

Keywords: Cecal Ligation and Puncture (CLP), exosomes, dexamethasone, Lung injury

The Immunoregulatory Property of Mesenchymal Stem Cells in Crocin Treatment by Modulation of microRNA-155, microRNA-21, microRNA-23b, microRNA-126a, and the Expression of their Targets.

Zeinab Rostami[1], Mohsen Naseri[2], Mohsen Khorashadizadeh[2], Mansoore Saharkhiz[3], Malaksima Ayadilord[3]

1. Student research committee, Mazandaran University of medical sciences, Mazandaran, Iran
2. Cellular and molecular research center, Birjand University of medical sciences, Birjand, Iran.
3. Student research committee, Birjand University of medical sciences, Birjand, Iran

Background: Mesenchymal stem cells (MSCs) are very interesting in clinical applications for immune disorders because of immunomodulation function in these cells. MSCs are present in different tissues although there are certain variations between them in their gene expression profile, immune modulation activity, and the secretion of factors. A lot of researches have currently been undertaken to enhance the immunomodulation function of MSCs and to pick an excellent type of MSC for clinical approaches. MicroRNAs (miRNAs) are small non-coding RNAs that adjust immunomodulation mechanism of MSC cells. Crocin is a carotenoid chemical compound that has anti-oxidant and anti-inflammatory and immunomodulatory effect that has been used for medicinal purposes. This study aims to assess the immunomodulatory related miRNAs expression and their target genes in both adipose derived stem cells (Ad-SCs) and dental pulp stem cell (DP-SCs) in the presence or lack of Crocin.

Methods: Ad-SCs from adipose tissue were extracted and DP-SCs were extracted from dental pulp and then treated with Crocin. The expression of 4 selected immunomodulatory-related micro-RNAs (i.e.-126, -21, -23, and-155) and these targets were assessed by RT-PCR in two MSCs.

Results: Our findings revealed that miRNA-23 and miRNA-126 up-regulated with Crocin treatment in MSCs and down-regulated miRNA-21 and miRNA-155 in the other side. Moreover, this experiment indicated that Crocin could suppress the PI3K/Akt1/Akt2/NFKB/relA expression in DP-SCs and decreased PI3K/Akt2 expression in Ad-SCs. As noted, these immuno-regulatory effects of Crocin were higher in DP-SCs than in Ad-SCs.

Conclusion: Crocin could suppress the PI3K/Akt1/Akt2/NFKB/relA genes expression by decreasing the expression of miRNA-23 and miRNA-126 or by increasing the expression of miRNA-21 and miRNA-155 that play a function in the immune regulation pathways in MSCs. Our findings can give an understanding of the mechanisms by which Crocin controls the immunomodulatory feature of MSCs. In addition, DP-SCs are a better immunomodulator in Crocin treatment than Ad-SCs. It may be helpful for MSCs selection in modulation or treatment of autoimmune disorders.

Keywords: mesenchymal stem cell (MSCs), micro-RNA (miRNAs), immunoregulatory or immunomodulatory, Crocin, Compare.

The Immunosuppressive Effects of Silymarin on Th17 Cells Representing a Potential as an Adjuvant Therapeutic Tool in MS Patients

Reza Hajiasghar-Sharbaf[1]

1. Isfahan University of Medical Science, Isfahan, Iran.

Background: Th17 cells play a critical role in the immune pathogenesis of multiple sclerosis. Despite using immunosuppressive drugs such as interferon β (IFN-β) to reduce the inflammatory response in multiple sclerosis (MS), none of these medications are followed by definitive treatment. In the present study, we aimed to consider the immunomodulatory effect of silymarin on Th17 cells isolated from newly diagnosed and IFN-β treated MS patients.

Methods: Blood-derived Th17 cells from MS patients were co-cultured in the presence of silymarin (50, 100, and 150 µM) for 48, 72, and 120 hours. Th17 proliferation was assessed by flow cytometry, and ROR-c gene expression was evaluated by real-time PCR respectively. Also, IL-17 production was determined by the ELISA method.

Results: We found that silymarin reduced Th17 cell proliferation in two groups compared to DMSO control. Besides, IL-17 production and ROR-c gene expression were decreased significantly in the presence of silymarin time and dose-dependently. This study is the first to indicate the effect of silymarin on Th17 cells.

Conclusion: Together, our findings suggest that silymarin's regulatory impact as a natural compound in suppressing Th17 cells could be of value in depressing the long-term consequences of inflammation in autoimmune diseases such as MS.

Keywords: Multiple Sclerosis, Silymarin, Interferon β therapy, Immunosuppressive

The Platelets in the Products of Leukocyte-rich PRP (LR-PRP) and Leukocyte-poor PRP (LP-PRP) Inhibit the Proliferation of Peripheral Blood Mononuclear Cells (PBMCs)

Somaye Karimi[1], Mitra Rafiee[2]

1. Department of Immunology, School of Medicine, Birjand University of Medical Sciences, Birjand
2. Cellular and Molecular Research Center, Department of Immunology, Birjand University of Medical Sciences, Birjand

Background: Platelets (PLTs) have been recently recognized as immunoregulatory cells. Upon activation, PLTs release cytokines, chemokines, growth factors, and PLT-derived microparticles (PMPs) and express activation molecules in the membrane surface as P-selectin (CD62P) or CD40L. Soluble factors released by PLTs can modulate leukocyte function. PRP is derived from autologous blood centrifugation with a primary focus on concentrated platelets above baseline. There are two types of PRPs including leukocyte-rich PRP (LR-PRP) and leukocyte-poor PRP (LP-PRP). The purpose of this study was to evaluate the effect of PLT dose-dependent manner, on the inhibit cellular proliferation of peripheral blood mononuclear cells (PBMCs).

Methods: Isolated PBMCs from 20 healthy volunteers were stimulated by PHA. LR-PRP and LP-PRP were prepared in a two-step centrifugation process following a protocol. PLTs and PBMCs were co-cultured for 48, and 72 h in 5% CO2 at 37°C. Then, cell viability was evaluated by the MTT test.

Results: Both products of PRP can inhibit cell proliferation in a dose-dependent manner ($P \leq 0.05$).

Conclusion: The present study demonstrated that PRP regulates PBMC function, decreasing and inhibiting cellular proliferation.

Keywords: Platelets (PLTs), Leukocyte-rich PRP (LR-PRP), Leukocyte-poor PRP (LP-PRP), Peripheral blood mononuclear cells (PBMCs)

The Relationship Analysis of microRNA-146a rs2910164 Polymorphism with Risk of Colorectal Cancer in Province of South-Khorasan, Iran

Mansoore Saharkhiz[1], Tahmine Tavakoli[1], Tahereh Fakharian[1], Ehsan Soltaninejad[1], Mohsen Naseri[1]

1. Birjand University of Medical Sciences, Birjand, Iran

Background: The microRNA-146a (miR-146a) is one of the common important miRNAs-related to immune/inflammatory systems. Under inflammatory conditions, the increased expression of this miR regulates inflame responses by earmarking and suppressing many effectors including TNF, TNFR, IRAK-1 & 2, IRF-3 & 5 and also boosting the Treg cells population and expression of IL-10. Recently, it is reported the association of rs291016 polymorphism in miR-146a gene with risk of different neoplasms such as CRC. In existent study, we have investigated role of this polymorphism in CRC risk for the first time in the province of South Khorasan, east of Iran.

Methods: In this research, the 54 people with CRC and 116 healthy people are selected from this province. After collecting blood samples and extracting genomic DNAs, the genotyping is conducted by PCR-RFLP technique. Finally, the data is analyzed using the SPSS program (16 version).

Results: After the data analysis and computing of odds ratio (OR) and P-value, it is detected the statistically important connections in comparison between rs291016 GG genotype and CC genotype (OR= 1.594, 95% CI=1.380–1.841, $p< 0.001$), GG genotype versus CC + CG genotypes (OR= 1.681, 95% C= 1.447–1.954, $p<0.001$), G-wild allele compared to C-mutant allele (OR= 1.296, 95% CI= 1.208–1.390, $p< 0.001$) among case and control groups.

Conclusion: The miR-146a rs2910164 GG genotype and G allele can be suggested as risk factors for occurrence of CRC in the studied patients.

Keywords: Colorectal cancer, MicroRNA-146a, Polymorphism, Immunoregulatory sequence

The Role of Autoantibodies against Myelin-Associated Glycoprotein in Schizophrenia

Marziyeh Soltani[1], Nader Bagheri[2], Pezhman Beshkar[3], Kobra Mokhtarian[3]

1. Students Research Committee, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
2. Department of Immunology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
3. Department of Laboratory Sciences, School of Allied Medical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran

Background: Schizophrenia (SCZ) is a severe psychological disorder associated with various positive and negative symptoms and cognitive disorders. In schizophrenia, a decrease in the molecules involved in the tight junction (Claudin5, Cadherin5) and an increase in adhesion molecules (ICAM1, VCAM1) can lead to in the decline in the blood–brain barrier (BBB) integrity and increased permeability. Abnormalities in the BBB can lead to immune response and autoimmune conditions. Therefore, investigation of autoantibodies that react against brain proteins is considerable. Myelin-associated glycoprotein (MAG) produced by oligodendrocytes contribute to myelin sheath formation. MAG plays an essential role in the development and function of the nervous system, so degenerative disorders are associated with this glycoprotein. The aim of this study was to investigate the role of autoantibodies against MAG in patients with SCZ.

Methods: A total of 40 patients with SCZ and 40 healthy subjects were enrolled in this study. Antibodies against MAG in patients with SCZ were measured using the indirect immunofluorescence assay and analyzed in ImageJ program. The chi-square ($\chi 2$) test or Fisher's exact test were used to compare the frequency of autoantibodies between patients with SCZ and control group.

Results: Among 40 SCZ patients, 3 (7.5%) from patients showed serum IgG antibody against MAG. These antibodies were seen in the white matter of the cerebellum against myelin sheath. However, the increase in the serum level of anti-MAG IgG in patients with SCZ was not significant compared to the control group (p= 0.241).

Conclusion: The increase in the serum level of autoantibody against MAG in patients with SCZ was not significant compared to the control group. Nevertheless, it is suggested to investigate this autoantibody in a larger statistical population. Dysregulation of the immune system and the production of autoantibodies can impair the function of the nervous system and affect the development of SCZ. Therefore, autoantibody testing is recommended in people with a history of mental illness as a screening and early diagnosis.

Keywords: Schizophrenia, Immune system, Autoantibodies, Myelin-associated glycoprotein, Pathophysiology

The Role of Inflammatory Mediators in the Pathogenesis of Periodic Fever, Aphthous Stomatitis, Pharyngitis and Cervical Adenitis (PFAPA) Syndrome

Daryush Purrahman[1], Lukasz A Poniatowski[2], Piotr Wojdasiewicz[3], Mohammad-Reza Fathi[4], Homayon Yousefi[1], Elena Lak[5], Mohammad-Reza Mahmoudian-Sani[6], Fatemeh Talebizadeh[7]

1. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
2. Department of Neurosurgery, Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland.
3. Department of Biophysics, Physiology and Pathophysiology, Faculty of Health Sciences, Medical University of Warsaw, Warsaw, Poland.
4. Department of Pediatric Neurology, Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5. Department of Internal Medicine, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
6. Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
7. Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Background: As a recurrent disease, periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome is characterized by episodes of febrile attacks and is often prominent in children under five years of age. However, the etiology of this condition has not been fully understood yet.

Methods: The search in the extensive literature of peer-reviewed articles published from the inception to December 2021 was conducted to identify the relevant studies, using the electronic databases of MEDLINE/PubMed, Embase, Scopus, the Cochrane Library, and the Web of Science.

Results: The analysis of complex relationships indicates that inflammatory factors, such as various cytokines and acute-phase proteins (APPs), play leading roles in the pathogenesis of this disease. Accordingly, this article summarizes the current state of knowledge to explain the mechanisms involved in inflammatory responses among patients with PFAPA syndrome and investigate its role in the pathogenesis of this disease. Moreover, the possibilities for further implementation of new therapeutic strategies are pointed out.

Conclusion: It is concluded that some pathophysiological processes are associated with immune dysregulation, which itself may be secondary to environmental factors, genetic background, and underlying diseases, including latent infections that multiply inflammatory mediators. Elevated inflammatory markers similarly play a significant part in the clinical outcomes of this condition, whose pyrogenic nature is the reason for the development of episodes of febrile attacks in the population of patients suffering from PFAPA syndrome.

Keywords: Acute phase protein, Cytokine, Fever, Inflammation, PFAPA syndrome

The Th17/Treg Imbalance and Related Cytokines as a New Sight in Pathogenesis of Idiopathic Membranous Nephropathy

Roza Motavalli[1], Mehdi Yousefi[2], Jalal Etemadi[3], Mohammad Sadegh Soltani-Zangbar[4], Farhad Motavalli Khiavi[5], Navid Shomali[6], Mohammadreza Sadeghi[1]

1. Department of Molecular Medicine, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Kidney Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
4. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
5. . Department of Virology, Pasteur Institute of Iran, Tehran, I.R. Iran
6. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Idiopathic membranous nephropathy (IMN) is main cause of nephrotic syndrome in adults determined by the deposition of autoantibodies on the subepithelial of the glomeruli capillary wall. Th17/regulatory T (Treg) balance plays prominent role in the regulation of autoimmunity.

Methods: In this study, we aimed to investigate the percentage of Th17 and Treg cells via flow cytometry. mRNA expression and protein levels of related master transcription factors and cytokines were assessed using real-time PCR and western blot analysis in mononuclear cells of peripheral blood of 30 patients with IMN and 30 healthy control before treatment.

Results: No considerable difference was observed in Th17 cell percentage, RORɣt, STAT3, IL-17, and IL-23 in mRNA expression and protein levels while IL-21, IL-4, and IL-10 had remarkable augmentation in mRNA expression and protein levels in peripheral blood mononuclear cells of IMN cases. Frequency of Treg cells mRNA expression FOXP3, and TGF-β expression illustrated decrease in IMN patients compared to the control group.

Conclusion: Our study demonstrate that Th17 cells themselves might not be involve in the pathogenesis of newly diagnosed patients with IMN; however, in this regards reduction of T reg cells and augmentation of Th17/Treg ratio might indicate a role in the pathogenesis of IMN before treatment.

Keywords: Regulatory T cell, T Helper 17 Cell, Primary membranous Nephropathy, Signaling pathway.

IL-10 and microRNAs 25, 29 as Potential Biomarkers for Coronary Artery Disease Related to Type 2 Diabetes Mellitus

Motahare Hatami[1], Nayere Askari[2]

1. Department of Cardiology, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran

Background: Over the past three to four decades, T2DM, a chronic, low-grade inflammatory disease, has increased dramatically in prevalence among adolescents and young adults. People with T2DM are more likely to develop coronary artery disease (CAD), and over half of them die from cardiovascular disease. Both T2DM and CAD are complex, multifactorial diseases with genetic, epigenetic, and environmental backgrounds, and both conditions have been hypothesized to share a common pathogenesis. Little is known about the role of IL-10 in the severity of coronary artery disease (CAD) in patients with diabetes. Recent studies have shown an important interaction between interleukin 10 (IL-10) and microRNAs. There are a number of microRNAs that are directly able to regulate IL-10 post-transcriptionally. On the other hand, IL-10 is able to regulate microRNAs. Identifying patients in the early stages of the disease is crucial for prevention and adequate treatment.

Methods: This is a case-control study conducted on 65 angiography-confirmed CAD patients and 45 controls with normal angiography (Non-CAD). Patients in both groups were examined and classified according to their history of diabetes. PBMC expressions of miR-25 AND miR-29 were evaluated by real-time PCR. The plasma concentration of IL-10 was measured by ELISA.

Results: Interleukin 10 plasma levels have a significant negative relationship with diabetes and the severity of CAD. Interleukin-10 plasma level among diabetic patients with CAD was significantly lower than those without CAD (p<0.05). In addition, a positive correlation was found between the expression of miRNA-29 and IL-10. Conversely, negative correlations were observed between miRNA-25 and IL-10 levels.

Conclusion: Overall, the data suggest as a preliminary study that interleukin 10 and miR-25, and miR-29 may be potential biomarkers for coronary heart disease in the context of diabetes. Further studies are needed to examine the reproducibility of these findings.

Keywords: IL-10, miR-25, miR-29, Coronary artery disease, Type 2 diabetes mellitus.

Innovative Technologies in Immunology

A Novel Fc-Engineered Anti-HER2 Bispecific Antibody with Enhanced Anti-Tumor Activity

Mehdi Mohammadi[1], Mahmood Jeddi-Tehrani[2], Forough Golsaz-Shirazi[3], Mohammad Arjmand[4], Fatemeh Torkashvand[5], Tannaz Bahadori[6], Mohammad Ali Judaki[6], Fariba Shiravi[6], Hengameh Ahmadi Zare[7], Farzaneh Notash Haghighat[2], Maryam Mobini[7], Fazel Shokri[7], Mohammad Mehdi Amiri[7]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3. Tehran University of Medical Sciences, Tehran, Iran
4. Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran
5. Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
6. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
7. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: HER2 overexpression has been demonstrated in a variety of cancers. Targeted therapy with anti-HER2 monoclonal antibodies (mAbs) has been approved as a therapeutic modality. Despite the efficacy of mAbs in tumor treatment, many patients do not benefit from this therapeutic platform. Fc engineering is a common approach to improve the efficacy of therapeutic mAbs. We have recently developed a potent anti-HER2 bispecific mAb (BsAb), BiHT, constructed from trastuzumab and our novel humanized anti-HER2 mAb, hersintuzumab. Here, we aimed to modify the Fc of BiHT to improve its therapeutic efficacy.

Methods: Two amino acids of the BiHT Fc were modified to improve its ADCC function. ELISA and flowcytometry were used to evaluate the binding profile of BsAbs. 3H-thymidine incorporation and LDH release assays were employed to determine the effect of antibodies on tumor cell proliferation and ADCC function, respectively. HER2 downregulation and signaling pathways were assessed by Western blotting. Nude mice were used for in vivo xenograft tumor models.

Results: The Fc-engineered BiHT (MBiHT) bound to recombinant HER2 and its subdomains with an affinity similar to BiHT. It also recognized native HER2 on different cell lines, inhibited their proliferation, downregulated HER2 expression and suppressed downstream signaling pathways similar to BiHT. Compared to BiHT, MBiHT displayed enhanced ADCC activity against various tumor cell lines. It also inhibited the growth of ovarian xenograft tumor in nude mice more potently than BiHT.

Conclusion: Our findings suggest that MBiHT could be a potent therapeutic candidate for treatment of HER2-overexpressing cancer types.

Keywords: Cancer immunotherapy, HER2, Bispecific antibody, Fc engineering, ADCC

Alleviation of Psoriasis Inflammation in The Imiquimod Induced Psoriasis Mouse Model Using Anti-IL-17A And Anti-TNF-α Ssdna Aptamers

Mohammadreza Dashti[1], Mojtaba Sankian[1]

1. Immunology Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: In psoriasis pathogenesis, IL-17 and TNF-α can synergistically affect the endothelial, keratinocytes, and immune cells to release TNF-α, IL-17A, IL-6, IL-1β, CCL20, and S100a. In recent years, biological agents such as monoclonal antibodies have been approved and administered for psoriatic patients. Despite the good efficacy of antibodies in the treatment of psoriasis, disadvantages such as high expensiveness, high immunogenicity, and time-consuming of these agents reduce their application in psoriasis treatment. IL-17 is a crucial protein in psoriasis pathogenesis that induces the hyper-proliferation of keratinocytes. Up to now, several antibodies against IL-17 have been approved for psoriasis treatment.

Methods: Aptamers, as single-stranded DNA or RNA molecules, have advantages such as time-saving, less risk for immunogenicity, good penetration into tissues, thermo-stability, and cost-effectiveness compared to antibodies. In this regard, in the present research, we assessed the therapeutic effects of M7 anti-IL-17A and T1-T4 dimer anti-TNF-α ssDNA aptamers in the imiquimod (IMQ)-induced psoriasis animal model. Vaseline (Vas) and 5% IMQ cream were prescribed on the right ear of BALB/c mice as Vas control and IMQ induced psoriasis model groups, respectively. Moreover, hydrogel-containing anti-IL-17A and anti-TNF-α aptamers were topically prescribed to the mice's ears 10 minutes before IMQ cream treatment. The psoriasis area severity index (PASI) score was used to evaluate psoriasis intensity. Sections of mice ears were stained with H & E for histopathology analysis. Moreover, mass, size, and cell number of spleens were measured. Axillary lymph node cells were cultured in each group, and after stimulation with 3% phytohemagglutinin (PHA) for 24 hours, the IL-17 level was determined in cell culture supernatants using ELISA. Additionally, the mRNA levels of IL-17A, IL-1β, STAT3, and S100a9, were evaluated with quantitative RT-PCR in mice treated ear.

Results: The anti-IL-17A and anti-TNF-α ssDNA aptamers significantly ameliorated IMQ-induced cumulative PASI score ($p < 0.05$). In addition, the IL-17A, IL-β, STAT3, and S100a9 mRNA expression level and IL-17 protein level decreased in the anti-IL-17A aptamer treated group compared to the IMQ group ($p < 0.05$). Also, in the anti-TNF-α groups, the IL-17A, STAT3, and S100a9 mRNA levels were significantly lower than the IMQ group ($p < 0.05$).

Conclusion: Despite promising results of anti-IL-17A and anti-TNF-α ssDNA aptamers in reducing psoriatic inflammation, the mix of these aptamers had lower efficacy. According to our findings, these aptamers seem to be a prospective candidate for treating psoriatic inflammation by inhibiting IL-17A and TNF-α activity.

Keywords: Psoriasis, Aptamer, TNF-α, IL-17A, Imiquimod, Animal model, Therapy

Ameliorative Effects of Gold And Copper Nanoparticles Synthesized Using the Aqueous Extract of Allium Saralicum on Ulcerative Colitis in Rats

Mahtab Pourkamalzadeh[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[2], Naser Abbasi[2]

1. Department of Microbiology, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.
2. Biotechnology and Medicinal plants Research Center Ilam University of Medical Sciences, Ilam, Iran

Background: Ulcerative colitis is a common cause of gastrointestinal diseases in developed countries. This disease causes disability, especially in Adults become young and impose a great economic and social burden on society. This study was designed to evaluate the effects of gold and copper nanoparticles of Allium saralicum aqueous extract on ulcerative colitis in rats.

Methods: 40 male Wistar rats were divided into five groups: untreated control, positive control group (acetic acid-induced ulcerative colitis), the aqueous extract of Allium saralicum treated group (200mg/kg/day) , the gold nanoparticles of Allium saralicum (0/5mg/kg/day), the copper nanoparticles of Allium saralicum (0/5mg/kg/day) and treated group with prednisolone(6mg/kg/day). After 10 consecutive days. The rats were dissected and blood, intestine samples of them collected for hematological, biochemical, Immunology, and gross parameters analysis. The data were analyzed using one-way ANOVA followed by Duncan post hoc test.

Results: The results showed that the reduction of tissue myeloperoxidase, Nitric oxide, Malondialdehyde production in gold nanoparticles group compared to peridenozolone was statistically significant does not have. The silver nanoparticles (AgNPs) of aqueous extract Matricaria chamomilla could significantly ($p \leq 0.05$) decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the Other groups. Also, the silver nanoparticles (AgNPs) of aqueous extract Matricaria chamomilla extract prevented significantly ($p \leq 0.05$) small, medium and large intestine ulcers as compared to the other groups.

Conclusion: These results demonstrated the silver nanoparticles of Allium saralicum aqueous extract as Suitable chemical composition is a promising strategy to improve the inflammation in a rat model of ulcerative colitis.

Keywords: Gold Nanoparticles of Allium Saralicum Aqueous Extract; Ulcerative Colitis; Acetic Acid

Applications of AI-Based Models in Managing Systemic Lupus Erythematosus

Benyamin Gheiji[1]

1. Student Research committee, Mashhad University of Medical Science, Mashhad, Iran

Background: Despite decades of research on systemic lupus erythematosus (SLE), diagnosis and treatment of this highly heterogenous disease is still challenging. Recent studies have indicated that machine learning and deep learning techniques have shown significant potential in helping to improve the diagnosis, treatment, and management of SLE. The purpose of this study is to review some of the applications of AI-based models in handling SLE.

Methods: According to our keywords "Systemic Lupus Erythematosus, machine learning, deep learning", we searched PubMed, Google scholar, and Scopus.

Results: Diagnosis: SLE can be detected through machine learning and deep learning models, which analyze large amounts of patient data such as clinical features, laboratory results, and imaging data. Disease activity prediction: The activity of the disease can also be predicted using machine learning and deep learning. These models are capable of considering a range of factors influencing disease activity, including drug use, laboratory data, clinical symptoms, and patient demographics. This would make it easier for doctors to anticipate the possibility of disease flare-ups and adjust treatments as necessary. Treatment response prediction: It is also possible to anticipate how SLE patients would react to various therapies using machine learning and deep learning models. These models can discover variables that might affect a patient's response to treatment through the analysis of patient data, and they can offer clinicians individualized treatment suggestions. Drug discovery: Machine learning and deep learning can also be applied to drug discovery for SLE. These models analyze huge datasets of chemicals to figure out what drug candidates could target SLE's underlying mechanisms.

Conclusion: Overall, the use of machine learning and deep learning techniques has the potential to advance our ability to manage SLE. These techniques could help doctors diagnose patients earlier, personalize their care, forecast how their diseases will progress, and find novel medicines.

Keywords: Systemic Lupus Erythematosus, Machine Learning, Deep Learning

Chaperon Co-Expression to Improve Soluble Expression of A Bi-Specific Tandem SCFV Targeting CTLA-4 and PD-1

Nafiseh Saadat Mousavi[1]

1. Isfahan University of Medical Sciences, Isfahan, Iran.

Background: During the production of dual antibodies for use in the treatment of diseases, especially cancer, the accumulation of proteins in the form of inclusion is one of the main problems. In order to reduce the accumulation of insoluble protein, the expression of recombinant protein with chaperones is used. In this study, we examined concomitant expression with chaperone to improve antibody solution expression tandem SCFV against two immune system checkpoints CTL-4 and PD-1.

Methods: Single gene SCFV transformation and simultaneous transformation of plasmids contains chaperon and SCFV into cells E. coli BL21 (DE3) was done. Protein expression was confirmed by western blotting. Also, the expression of soluble and insoluble proteins desired by the techniques ds-page the conditions for optimizing protein production with temperature changes and inductance concentration changes were investigated.

Results: The desired protein expression was investigated by western blot method. As can be seen, the high expression of the target protein in bacteria (E. Coli BL21 (DE3) after induction by IPTG (presence of 55 kDa band) and the lack of expression of this protein before induction. the optimal conditions for the expression of soluble and insoluble proteins was determined by changing temperature and concentration IPTG. The results showed that the expression of soluble protein at 25 ° c was higher than other temperatures. Also, the concentration of 1 mm of the inducer showed the highest expression of soluble protein. Purification of proteins by denaturation method: Insoluble proteins are purified by denaturation. Since most of the expressed protein was insoluble at 37 ° C, purification was performed at this temperature under denaturation conditions using a nickel chromatographic column and in a denaturing elustion buffer. Different purification steps were confirmed by protein analysis using SDS-PAGE electrophoresis. In order to determine the optimal conditions for the production of soluble and insoluble proteins, the conditions were investigated simultaneously with chaperone expression at temperatures of 25, 30 and 37 ° C with concentrations of 0.25, 0.5, and 1 mM IPTG. The results showed that the expression of soluble protein at 30 ° c and the expression of insoluble protein at 25 ° c was higher than other temperatures. Also, both states of the desired protein (soluble and insoluble) at a concentration of 1 mm IPTG were higher than other concentrations. Simultaneous expression with chaperone at 30 degrees is the best condition for protein solution expression.

Conclusion: Simultaneous expression of dual antibodies SCFV in contrast, chaperone has improved the average solubility of the antibody and can be used to improve the expression of soluble protein in other studies.

Keywords: Bi-specific antibody, Chaperone, Co-expression, CTLA-4 ،PD-1

Comparative Study Of Anti-Inflammatory Cytokine Levels In Rats Treated With Hottentotta Saulcyi And Mesobuthus Eupeus.

Mohammad Nemati[1], Mohammad khosravi[2], Fatemeh Salabi[3], Hedieh Jafari[3]

1. Assistant Professor, Department of Venomous Animals and Anti-venom Production, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Ahvaz, Iran.
2. Department of Pathobiology, faculty of veterinary medicine, shahid chamran university of Ahvaz, Ahvaz, Iran.
3. Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Ahvaz, Iran.

Background: Scorpion venom contains various biological compounds. Clinical symptoms in individuals and laboratory animals exposed to scorpion venom depend on the response of the host immune system.

Methods: This comparative study aimed to evaluate the expression of anti-inflammatory cytokine IL-10 in rats treated with Hottentotta saulcyi and Mesobuthus eupeus scorpion venom. The venom was obtained from the Razi Vaccine and Serum Research Institute of Ahvaz branch. After determining the H. saulsyi and M. eupeusvenom LD50, the rats were divided in 3 groups of test and control (n-12).The test group received 1/3 LD50 dose in 0.5 ml of physiological serum by subcutaneous injection per rat. The exact amount of physiological serum was injected into the control group. After that, cardiac blood samples were taken from rats at 0, 4, 24 and 72 hours after anesthesia. After serum preparation, the levels of IL-10 cytokine were measured in three groups using ELISA assays.

Results: The obtained LD50 equaled 1.01 mg/kg (H. saulsyi) and 11/5mg/kg (M. eupeusvenom) of the rat's body weight. Four hours after experimentally envenomation, the serum levels of IL-10 in rats treated with H. saulsyi were significantly increased compared to the control group (P <0.05); but in the taken samples 24 hours after the treatment, there was no significant difference compared to the control group. During 72 hours, the level of these cytokines decreased in the treatment group compared to the control group. the serum levels of IL-10 in rats treated with M. eupeusvenom the serum level of interleukin 10 at 4 hours after injection of the venom was significantly increased compared to its value at zero time (p <0/05).observed that the amount of interleukin 10 at 24 hours after injection of the venom was reduced compared to the amount at 4 hours and this difference was significant (p < 0/05).amount of interleukin 10 at 72 hours after injection of the venom, compared to its value at 24 hours after injection of the venom decreased and reached a stable state, but this difference was not significant (p> 0.05).

Conclusion: Changes in anti-inflammatory cytokines levels during scorpion stings can be used as a novel clinical finding to assess patients' status and perform appropriate therapeutic interventions to reduce scorpion sting complications.

Keywords: anti-inflammatory cytokine Hottentotta saulcyi Mesobuthus eupeus scorpion venom

Competitive Effect of Overexpressed C-terminal of Snail-1 (CSnail) is a Promising Strategy in Control of the Growth and Metastasis of Melanoma Cells

Sadegh Paydari Rostami[1], Negar Moghare Dehkordi[1], Yazdan Asgari[1], Mohammad Reza Bolouri[2], Nasrin Shayanfar[3], Reza Falak[2], Gholam Ali Kardar[5]

1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran.
3. Department of Pathology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
4. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran
5. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Background: Epithelial-to-mesenchymal transition (EMT) plays role in the invasion and metastasis of cancer cells. During this phenomenon, Snail can promote tumor progression by upregulating mesenchymal factors and downregulating the expression of pro-apoptotic proteins. Therefore, interventions on the expression rate of Snail may show beneficial therapeutic applications.

Methods: In this study, the C-terminal region of Snail1, capable of binding to E-box genomic sequences, was subcloned into the pAAV-IRES-EGFP backbone to make complete AAV-CSnail viral particles. B16F10 as a metastatic melanoma cell line, with a null expression of wild type TP53 was transduced by AAV-CSnail. Moreover, the transduced cells were analyzed for in-vitro expression of apoptosis, migration, and EMT-related genes, and in-vivo inhibition of metastasis.

Results: In more than 80% of the AAV-CSnail transduced cells, the CSnail gene expression competitively reduced the wild-type Snail functionality and consequently lowered the mRNA expression level of EMT-related genes. Furthermore, the transcription level of cell cycle inhibitory factor p21 and pro-apoptotic factors were promoted. The scratch test showed a decrease in the migration ability of AAV-CSnail transduced group compared to control. Finally, metastasis of cancer cells to lung tissue in the AAV-CSnail-treated B16F10 melanoma mouse model was significantly reduced, pointing out to prevention of EMT by the competitive inhibitory effect of CSnail on Snail1 and increased apoptosis of B16F10 cells.

Conclusion: The capability of this successful competition in reducing the growth, invasion, and metastasis of melanoma cells indicates that gene therapy is a promising strategy for the control of the growth and metastasis of cancer cells.

Keywords: Adeno-associated virus, Epithelial mesenchymal transition, Melanoma mouse model, Metastasis, Snail, Tumor suppressor protein p53

CRISPR-Cas9 for Cancer Therapy; Systematic review

Pouria Ahmadi Simab[1], Negar Niyazmand[2], Soheil Sadr[3], Hassan Borji[4]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
2. Department of English Language and Literature, Faculty of Letters and Humanities, Ferdowsi University of Mashhad, Iran
3. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
4. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Initiated by a variety of complex mutations, such as oncogenes activating and tumor suppressors inhibiting, cancer is a leading cause of death. Gene editing with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has attracted considerable interest, enabling research into the mechanism of cancer growth and developing precise therapies. This study aims to utilize the CRISPR-Cas9 technique for cancer research, its challenges, and possible solutions, emphasizing its potential for cancer therapy.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on CRISPR-Cas9 for cancer therapy from January 2000 to January 2023. Twenty affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: CRISPR/Cas9 is a genetically modified prokaryotic antiviral immune system that attacks viral infections and employs RNA-guided nucleases to remove foreign DNA. It consists of two sections: single-stranded guide RNA (sgRNA) and Cas9 endonuclease. Then, the sgRNA guides the Cas9 endonuclease to attack the desired gene's DNA with a specific sequence. The genome DNA is fixed by the double-stranded break (DNA-DSB) repair processes following separation. Human Lung tumors with molecular characteristics and histopathological can be induced in mice via chromosome rearrangement and CRISPR Cas9-mediated gene editing. Furthermore, CRISPR-Cas9 has been employed to prevent several breast cancer types.

Conclusion: The positive results given by these proven modifications indicate that in the future, chemists should take a riskier approach-research needs to examine what chemistry is best for developing CRISPR therapies and other in vivo techniques for fighting cancers.

Keywords: Breast cancer, CRISPR/Cas9, Lung cancer, Systematic review

Design and Preparation of Diagnostic Slides for Anti-neutrophil Cytoplasmic Antibodies (ANCA) by Indirect Immunofluorescence Method

Faezeh Shahba[1], Mohammad-Ali Assarehzadegan[1], Majid Khoshmirsafa[1,2*]

1. *Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of medical Sciences, Tehran, Iran,*
2. *Department of Immunology, Iran University of Medical Sciences, Tehran, Iran*

Background: One of the most important tests for the detection of antibodies against neutrophil cytoplasm in autoimmune vasculitis diseases is the Antineutrophil Cytoplasmic Antibodies (ANCA) test. The ANCA test is the first order in the initial diagnosis and screening of patients with suspected autoimmune diseases, including small-vessel vasculitis, as well as the follow-up of the patient's treatment, which uses indirect immunofluorescence assay (IFA) to detect the presence of antibodies against antigens in the cytoplasm of neutrophils. In addition to vasculitis, the evaluation of this antibody is used in other autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and inflammatory bowel diseases (IBD).

Methods: Using the Ficoll density gradient centrifugation and dextran solution, neutrophils were isolated from the peripheral blood of healthy. The purity and efficiency of the isolated cells were evaluated by Wright Giemsa staining, the neobar slide, and an automaticcost-effectivehine. Isolated neutrophils were fixed on slides using an ethanol fixative. At this stage, by selecting the best protocol, in terms of time and type of neutrophil fixation stage, they were fixed on the slide. In the next step, using positive and negative samples that have already been determined by ANCA with valid commercial kits (IVD certified), the slides containing fixed cells were fluorescent stained and compared with control samples.

Results: The number and purity of neutrophils isolated were evaluated by a Sysmex automatic cell counter. Depending on the peripheral blood neutrophils, the mean (±standard deviation) of the number of isolated neutrophils was 6000(±950) cells per microliter. According to white blood cell differential count, the mean purity of isolated neutrophils was 94.1%. Also, by Trypan blue staining, the percentage of viable cells was determined to be more than 85% before fixation. The results of ANCA indirect fluorescent staining on the prepared slides were completely consistent with the results of control samples.

Conclusion: The results of this study showed that the method used can be applied for the commercialization and production of ANCA slides. In this regard, in addition to optimizing the steps of separation and fixing the neutrophils, it is necessary to evaluate and optimize the stability of the prepared slides during storage and production in order to produce in large numbers.

Keywords: ANCA, Diagnosis slide, indirect immunofluorescence assay, Neutrophil, Vasculitis

Development of anti DLL4 Nanobody fused to truncated form of Pseudomonas exotoxin: as a novel immunotoxin to inhibit of cell proliferation and neovascularization

Rasoul Baharlou[1], Maryam Mohammadlou[1]

1. Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran

Background: Targeted tumor therapy is an attractive approach for cancer treatment. Delta-like ligand 4 (DLL4) is overexpressed in tumor vasculature and plays a pivotal role in tumor neovascular development and angiogenesis during tumor progression. Immunotoxins due to their superior cell-killing ability and the relative simplicity of their preparation, have great potential in the clinical treatment of cancer. The aim of this study was to develop a novel immunotoxin against DLL4 as a cell cytotoxic agent and angiogenesis maturation inhibitor.

Methods: In present study, an immunotoxin, named DLL4Nb-PE, in which a Nanobody as targeting moiety fused to the Pseudomonas exotoxin A (PE) was constructed, expressed and assessed by SDS-PAGE, western blotting, ELISA and flowcytometry. The functional assessment was carried out via MTT, apoptosis and CAM assays.

Results: It was demonstrated DLL4Nb-PE specifically binds to DLL4 and recognizes positive-DLL4 MKN cells. Cytotoxicity assays showed that this molecule could induce apoptosis and kill DLL4-positive MKN cells. In addition, it inhibited neovascularization in the chicken chorioallantoic membrane.

Conclusion: Our findings indicate designed anti-DLL4 immunotoxin has valuable potential for application to the treatment of tumors with high DLL4 expression.

Keywords: Immunotoxin, Pseudomonas exotoxin A, Nanobody, DLL4, Targeted tumor therapy

Discovery of Tissue Protein Biomarker in Bladder Cancer Patients with Mass Spectrometry-based Approach

Samira Tabaei[1], Mohammadreza Haghshenas[2], Ali Ariafar[3], Kambiz Gilani[4], Allan Stensballe[5], Shirin Farjadian[6], Abbas Ghaderi[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Urology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Integrative Oncology Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
5. Department of Health Science and Technology, Aalborg University, 9200, Aalborg Ø, Denmark
6. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: The aim of this study is to recognize the proteome comparison between non-muscle invasive (NMIBC), muscle invasive (MIBC), and adjacent non-cancerous tissues of bladder cancer patients (BC) using combined two-dimensional electrophoresis (2-DE) and mass spectrometry to identify differentially expressed proteins.

Methods: We compared the proteome of tissue samples of 42 UBC patients (NMIBC n=25 and MIBC n=17) using 2-DE followed by Liquid chromatography–mass spectrometry (LC–MS) system to identify differentially expressed proteins in NMIBC, MIBC, and adjacent non-cancerous tissues. Twelve differentially proteins expressed between NMIBC vs. MIBC were selected to identify by LC-MS. In silico analysis were performed to characterize function and biological pathways of selected proteins.

Results: Twelve differentially proteins expressed (over 2-fold, $p<0.05$) were identified by LC-MS. Nine proteins were significantly up-regulated in MIBC, while three proteins were significantly down-regulated. In silico analyses confirmed that peroxiredoxin activity, oxidoreductase activity, cadherin binding, and glutathione peroxidase activity were the most involved pathway.

Conclusion: We identified several alterations in protein expression and found the canonical pathways were correlated with the clinical outcomes that may be useful as promising biomarkers for early detection, monitoring, and progression of BC.

Keywords: Urothelial bladder cancer, Proteomics, Biomarker

Establishment of Stable CHO Cell Lines for Overexpression of whole-length CD20 Antigen

Niloufar Mohammadkhani[1], Masoumeh Rajabibazl[2]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: CD20 is a differentiation-related antigen exclusively expressed on the B lymphocytes' membrane. CD20 amplification is observed in numerous immune-related disorders, making it an ideal target for immunotherapy of hematological malignancies and autoimmune diseases. Monoclonal antibody (mAb)-based therapies targeting CD20 have a principal role in the treatment regimens. Since in hematopoietic cells, Fc gamma receptors mediate antigenic modulation, this study aimed to establish non-hematopoietic stable cell lines overexpressing whole-length human CD20 antigen as an in vitro model to be recruited in CD20-related studies.

Methods: CD20 gene was cloned into the transfer vector. The Lentivirus system was transfected to packaging HEK 293T cells and the supernatants were harvested. CHO-K1 cells were transduced using recombinant viruses and a stable cell pool was developed by the antibiotic selection. CD20 expression was confirmed at the gene and protein levels notably, simultaneous expression of GFP protein facilitated the detection of CD20-expressing cells.

Results: Immunophenotyping analysis of stable clones demonstrated that cells stably express CD20 antigen and the mean fluorescence intensity was significantly higher on the established cell clones than on negative control cells.

Conclusion: This study was the first report on using second-generation lentiviral vectors for the establishment of a non-hematopoietic cell-based system, which stably expresses full-length human CD20 antigen. Produced stable CHO cell lines with different levels of CD20 antigen are therefore nicely suited to be used for CD20-based studies, including binding and functional assays.

Keywords: CD20, immunotherapy, in vitro model, Chinese Hamster Ovary cell, lentiviral vector

Evaluation of Anti-Cancer Effect of A Diabody against PD-1 and CTLA-4 on Breast Cancer Cells

Nafise Moosavi[1]

1. Isfahan University of Medical Sciences, Isfahan, Iran.

Background: Many studies showed that co-targeting of immune checkpoints can improve the efficacy of cancer treatment. Different types of bispecific antibodies have been produced for cancer treatmen. One of the common types of bispecific antibodies is single chain antibodies such as diabody. Here, we aimed to evaluate the anticancer effect of a bispecific diabody against two immune checkpoints, PD-1 and CTLA-4.

Methods: Cytotoxicity of anti-PD-1/anti-CTLA-4 diabody against MCF-7 and MDA-MB-231 cells was evaluated using MTT method. Apoptotic effect of the diabody was detected using Annexin/propidium iodide method. The apoptosis induction was also checked by western blotting. The effect of protein on cell cycle was examined using flow cytometry.

Results: Cytotoxicity of the diabody against MDA-MB-231 cells was more than MCF-7. Cell survival at 400 nM concentration of the diabody was 42% for MDA-MB-231 and 69% for MCF-7. The result of the statistical test shows that the lethal effect of diabody and doxorubicin in these concentration, 25-50-100-200 nM and 21.8, 43.7, 81.5, 175 nM in MCF-7 with MDA-MB-231 with P-value less than 0.05 is significant. The diabody at concentration of 400 nM led to 31.7% apoptosis according to Annexin/propidium iodide method. The western blot analysis showed that the diabody at concentration of 400nM increased the ratio of Bax/Bcl-2 form 0.56 to 0.64. Cell cycle evaluation showed that the diabody led to cell cycle interruption, leading to cell cycle arrest in G2 and decrease S.

Conclusion: Our results indicate that the toxicity of this diabody was more in MDA-MB-231 (PD-L-1 overexpressing cell line) then MCF-7 (PD-L-1 low expressing cell line). Immune checkpoint inhibitors can improve breast cancer treatment particularly PD-L-1 overexpressing type. Conclusions: This diabody could be a potential anticancer agent and should be evaluated for more invitro and in-vivo experiments.

Keywords: Cancer, diabody, Immmun checkpoint, Cytotoxicity

Exosome-Induced Alternative Macrophages in Tissue Repaire in CLP-Induced Sepsis Mice

Sara Soufihasanabad[1], Zahra Mirsanei[2], Fatemeh Ahangari[2], Esmaeil Babaei[1], Seyed Mahmoud Hashemi[2], Sara Soudi[3]

1. Department of Animal Biology, School of Natural Sciences, University of Tabriz
2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences
3. Department of Immunology, Faculty of medical sciences, Tarbiat Modares University

Background: Considering that sepsis is an acute inflammation and also leads to tissue damage in the lung, liver and kidney, this study was designed to evaluate the effect of alternative macrophages induced by exosomes on the dynamics of tissue damage in the mouse sepsis model.

Methods: We used alternative macrophages induced by exosomes to characterize tissue damage in the lung, kidney, liver tissues, in a cecal ligation and puncture (CLP)-induced polymicrobial sepsis murine model.

Results: The CLP surgery significantly increased tissue damage in lung, liver and kidney In sepsis mice. Instead, less damage was observed in mice treated with alternative macrophages induced by exosomes.

Conclusion: Reduction of tissue damage indicates the positive effect of alternative macrophages induced by exosomes on inflammatory responses. Probably, alternative macrophages induced by exosomes reduce inflammatory mediators by producing anti-inflammatory cytokines and reducing nitric oxide production.

Keywords: Tissue damage, Alternative macrophages, Sepsis

Expression, and Evaluation of the Biological Activity of an Immunocytokine Composed of Mutant Interleukin 2 and Anti-vascular Growth Factor Receptor 2 (VEGFR2) Nanobody

Arezoo Beig Parikhani[1], Mahdi Behdani[1]

1. Venom and Biotherapeutics Molecules Laboratory, Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

Background: The off-target effects of IL-2 on different cell populations and causing severe side effects at high doses, as well as insufficient activation of antitumor effector cells at tolerable doses, have limited its use in cancer treatment. Through a decreased affinity for the IL-2R, mutant IL-2 molecules lessen toxicity and increase the interleukin's potential for therapeutic usage.

Methods: Creating antibody-cytokine fusion proteins (immunocytokine) to target IL-2 to the tumor microenvironment is another intriguing method to increase its half-life and effectiveness without associated damage. Due to their smaller size, the use of antibody fragments in this fusion may be more successful than the use of commercial antibodies. In this research, a novel immunocytokine known as VGRmIL2-IC—a fusion of mutant IL-2 with reduced affinity for CD25 and a specific nanobody against VEGFR2—was designed, produced, and its biological activity was assessed. We assessed and compared the expressed immunocytokine to unconjugated wtIL-2 and mIL-2 in terms of antigen binding, cell proliferation, IFN-secretion, and cellular cytotoxicity. Furthermore, pharmacokinetic characteristics were evaluated in vivo. Flow cytometry analysis revealed that mIL-2 has less affinity to the receptor subunit than wtIL-2. Additionally, VGRmIL2-IC molecule can specifically recognize and bind to VEGFR2 on cells similar to 3VGR19 nanobody. The findings demonstrated that VGRmIL2-IC has a lower function than wtIL-2 in terms of PBMC proliferation and stimulation of IFN- production by these cells, but it has a similar function to mIL-2 and a stronger cytotoxic ability than wIL-2.

Results: The antitumor activity of mIL-2 was significantly higher than that of wtIL-2 in in vivo tests, and VGRmIL2-IC's pharmacokinetic properties were better compared to those of both unconjugated IL-2 proteins.

Conclusion: Preliminary findings from this work indicate that VGRmIL2-IC may be a promising candidate for cancer therapy strategies; however, more research is required to fully understand the biopharmaceutical potential of this immunocytokine.

Keywords: Immunotherapy, Immunocytokine, Interleukin-2, Mutation, Nanobody, VEGFR2, Bioinformatics

From in-silico prediction to bench for changing membrane PD-1 expression to soluble; applying CRISPR/Cas9 strategy in Jurkat cells

Zeinab Yousefi-Najafabadi[1], Gholam Ali Kardar[2], Reza Falak[3]

1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Immunology, Asthma & Allergy Research Institute (IAARI), Tehran University of Medical Sciences, Tehran, Iran.
3. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: The membrane PD-1 is an inhibitory biomarker expressed on immune cells, binding to PD-L1/PD-L2 on tumor cells and resulting in T-cell exhaustion. The PD-1 expression could be knocked-out in T cells using CRISPR/Cas9 system to reverse the exhaustion. The soluble PD-1 is an active protein, expressed after exon-3 deletion through selective splicing. The sPD-1 has therapeutic biomarker value in TME during PD-1/PD-L1 inhibitory signaling pathway disruption. For blocking the mPD-1 expression and increasing sPD-1 in T cells, the exon deletion approach of CRISPR/Cas9 system could be used to delete exon-3 of pdcd1 gene.

Methods: In this study we used bioinformatic tools to define transmembrane domain of PD-1 protein and stimulation of the sPD-1 structure by removing the exon-3 sequence. The target splicing site of exon-3 in pdcd1 sequence was predicted for designing CRISPR/Cas9 sgRNAs. The dual-sgRNAs were cloned into lentiCRISPR-v2-puro vector and lentiviral particles were packaged in HEK-293t. The Jurkat cell line was used for lentiviral transduction to deletion of mPD-1 expression and increasing of sPD-1 expression. Sequencing analysis of pooled transduced Jurkat cells genome was done with TIDE tool to investigate the CRISPR effect. Flow cytometry and dot blot were used to confirm the change in expression pattern.

Results: The in-silico analysis defined that exon-3 is responsible for transmembrane domain expression in mPD-1 and the deletion of this location could express sPD-1. The DNA sequencing TIDE analysis of pooled transduced Jurkat cells showed random Indel in pdcd1 gene near sgRNAs targets with total efficiency above 85%. The Jurkat cells after activation had 70% mPD-1 expression in cell surface and 30% sPD-1 in cell supernatant. After lentiviral transduction and expression of dual sgRNA CRISPR/Cas9 in pooled Jurkat cells, the expression pattern was changed with about 10 % decrease of mPD-1 into sPD-1 form.

Conclusion: Our results confirmed that the expression pattern of PD-1 could be changeable using CRISPR/Cas9 system. In future studies, we will test the effect of this strategy on the survival and cytotoxicity of T cells. This construct could be used in CAR-T cells and TILs gene-immunotherapy to overcome their exhaustion.

Keywords: Exhaustion, PD-1, CRISPR/Cas9, Immunotherapy

Generation of TIM-3 inhibitor single domain antibody by phage display technique

Vida Homayouni[1]

1. Immunodeficiency Diseases Research Centre, Isfahan University of medical sciences

Background: T cell immunoglobulin and mucin domain 3 (TIM3) is one of the major inhibitory immune checkpoints expressed on T cells. Blocking the interaction between TIM3 and its inhibitory ligand galectin 9 may potentiate the effects of immunotherapy or overcome the adaptive resistance. Based on the unique features of nanobodies, we aimed to construct an anti-TIM-3 nanobody as an appropriate tool for manipulating immune responses for future therapeutic purposes.

Methods: We immunized a camel with TIM-3 antigen and then, synthesized a VHH phage: prepared library from its B cell's transcriptome using nested PCR. Library. Selection against TIM-3 antigen was performed in three rounds of panning. Using phage-ELISA, the most reactive colonies were selected for sub-cloning in soluble protein expression vectors. The Nanobody was purified and confirmed with a nickel-nitrilotriacetic acid (Ni-NTA) column, SDS-PAGE and Western blotting. A flowcytometric analysis was performed to analyze the binding and biologic activities of theTIM-3 specific nanobody with TIM-3 expressing HL-60 and HEK cell lines an explanation of the study design and experimental method.

Results: Specific 15kD band representing for nanobody was observed on the gel and confirmed with Western blotting. The nanobody showed significant specific immune-reactivity against TIM-3 with a relatively high binding affinity. The nanobody significantly suppressed the proliferation of TIM-3 expressing HL-60 cell line.

Conclusion: we successfully prepared a functional anti-humanTIM-3 specific nanobody with a high affinity and an anti-proliferative activity on an AML cell line in vitro.

Keywords: Heavy chain antibody, VHH, Nanobody, Phage display, T-cell immunoglobulin and -mucin domain 3

Inhibition of HER2 Dimerization by a Novel Recombinant Single Domain Antibody (Nanobody) Produced Using Phage Display Technique

Ahmad Najafi[1], Reza Valadan[2], Hossein Asgarian-Omran[3], Alireza Rafiei[4], Armin Akbar[5], Amir Kahrizi[6], Mohsen Tehrani[7]

1. Mazandaran University of Medical Sciences, Sari, Iran.

Background: Expression of HER family of receptors has been observed in many cancers. Dimer-dependent phosphorylation of HER2 receptor is a key event for the signal transduction of HER family of receptors which correlates with tumor invasion and metastasis. Therefore, a new generation of therapies based on dimerization domain inhibition, using monoclonal or fragment antibodies, has been introduced to overcome the resistance to therapeutic antibodies, such as Herceptin. Phage display antibody library method is a powerful technique for producing whole antibody molecules and antibody fragments. In this study, we produced a recombinant single domain antibody (sdAb) against the HER2 dimerization domain.

Methods: A recombinant phage was generated using the phage display method. Subtractive panning was performed on immobilized dimerization domain peptide sequence on sepharose 4B. Polyclonal and monoclonal phage cell-ELISA was used to confirm the binding of phages to target region of cells. Inhibition of HER2 dimerization by the produced recombinant phage was evaluated using dimerization inhibition test. Also, cell viability was evaluated using MTT assay.

Results: PCR fingerprinting results showed a uniform digestion pattern following the fourth round of panning that confirmed the success of subtractive panning. Based on the results of polyclonal phage ELISA, the fourth round of panning led to isolation of several dimerization domain reactive clones. A positive clone with the highest affinity was then selected by monoclonal phage ELISA and used for antibody expression. Moreover, cell-ELISA, MTT assay, and dimerization inhibition test confirmed the reactivity of the produced recombinant phage to dimerization domain of HER2.

Conclusion: We selected a clone with a high-binding affinity against HER2 dimerization domain and therefore, recombinant phage sequence of this clone is proposed to serve as targeting and/or treatment agents in the diagnosis and immunotherapy of HER2-positive tumors. Further studies on purified sdAb are needed to evaluate its in vitro and in vivo functions, via functional assays, to test whether it can be used in diagnosis and treatment.

Keywords: HER2, dimerization domain, recombinant phage, Phage display

Inhibition of Tumor Growth by Blockage of Adenosine Receptor

Vida Hashemi[1], Farhad Jadidi Niaragh[2]

1. Department of basic science, faculty of medicine, Maragheh University of medical Science, Maragheh, Iran
2. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: The expression of immune-checkpoint inhibitors molecules on infiltrating T cells is one of the chief reasons for the unsuccessfulness of various cancer immunotherapies. So, we decided to suppress one of the most important inhibitory checkpoint molecules expressed on tumor-infiltrating T cells (A2aR). Ligation of adenosine with A2aR significantly inhibit T cell responses against cancer cells.

Methods: we well designed NPs with high functional properties for delivery of A2aR siRNAs to cancer cells and then evaluated the potential of NPs loaded siRNA alone or in combination with DC-based vaccines in cancer treatment (4T1 and CT26 Mice tumors).

Results: It has been shown that that NPs loaded with A2aR siRNA molecules significantly inhibited the expression of A2aR target genes in tumor-infiltrating T cells.

Conclusion: Also noticeably induced anti-tumor immune responses, reduced tumor growth, suppressed Treg differentiation, and increased survival time in mice.

Keywords: Nanoparticle, siRNA, A2aR, Cancer therapy

Ozone Therapy Enhances Antitumor Activity in Mice Models of Colorectal Cancer

Reza Karbalaee[1], Morteza Izadi[2], Bahman Jalali Kondori[3], Ruhollah Dorostkar[4], Seyed Morteza Hosseini[5], Hadi Esmaeili Gouvarchinghaleh[6]

1. Students research committee, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Professor of Infectious Disease, Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Baqiyatallah Research Center for Gastroenterology and Liver Diseases (BRCGL), Baqiyatallah University of Medical Sciences, Tehran, Iran
4. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
5. Medicine, Quran and Hadith Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
6. Assistant Professor in Immunology, Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background: There is currently no known cure for cancer, despite the development of numerous new medications and cutting-edge surgical procedures. Treatment treatments including chemotherapy and radiation therapy can have fatal adverse effects and have a negative impact on a patient's quality of life. The technique of injecting ozone gas into a patient's body to treat a condition is known as ozone treatment. Even with the outstanding outcomes of ozone therapy for cancer, its effectiveness for malignancies is still being explored. Currently, the use of this technology in the treatment of cancers is a contentious subject. Investigating the effects of ozone in the treatment of colorectal cancer in mice is the goal of this study.

Methods: Colorectal cancer animal modeling was carried out by injecting 5×106 CT-26 cells (a colonic carcinoma cell line) into the left flank of female BALB/c mice. They were administered Ozone (40 g/ml, intraperitoneally, once every two days for three weeks) after witnessing the palpable tumor. To test the efficacy of the aforementioned therapies, half of the mice in each group were euthanized 10 days after the final treatment. The lifespan of the mice in each group's other half was examined to see how the treatment affected their longevity. An amount smaller than 0.05 was regarded as a significant statistical difference.

Results: According to the study's findings, mice who received the treatment had considerably better survival curves and a slower rate of tumor growth than tumor-bearing mice who only received a single agent's treatment or animals used as negative controls. They have, they did. Additionally, it markedly boosted the amount of lactate dehydrogenase and nitric oxide produced by tumor-bearing mouse spleen cell culture. Additionally, compared to the control group, it dramatically boosted the secretion of IFN-γ and decreased that of IL-4 and TGF-β in the spleen cell population.

Conclusion: The use of ozone in the treatment of colorectal cancer appears to be possible based on the findings of the current investigation.

Keywords: Colorectal cancer, Ozone, Nitric oxide, Lactate dehydrogenase

Production and Characterization of a New Anti-PD-L1 Monoclonal Antibody with Potential Diagnostic Application in Immunohistochemistry

Mansoure Mansouri[1], Mahdi Shabani[1], Mehdi Mohammadi[2], Mahmood Jeddi-Tehrani[3], Fazel Shokri4.Fatemeh Montazer[5]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences
2. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Monoclonal Antibody Research Center, Avesina Research Institute, Tehran, Iran.
4. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences.
5. Department of Pathology, Firoozabadi Clinical Research Development Unit, Iran University of Medical Sciences, Tehran, Iran.

Background: Programmed death-ligand 1 (PD-L1) is a checkpoint that regulates the immune response. The PD-L1 expression has been extensively studied in various cancer types. Its expression has been used as a biomarker to predict respond to checkpoint immunotherapy. In recent years, PD-L1 immunohistochemistry (IHC) testing has become an integral part of cancer diagnosis and treatment decision-making. Thus, the aim of this study is production and characterization of a new monoclonal antibody (mAb) to PD-L1 with diagnostic application in IHC.

Methods: Hybridoma method was used to produce mAb. For this purpose, female Balb/C mice aged 6-8 weeks were hyperimmunized by 5 times injection of commercial PD-L1 recombinant protein. Then, the mice were sacrificed and spleen cells and SP2/0 myeloma cells were fused using PEG. A stable clone was obtained with the limiting dilution method. After each subclone, ELISA screening test and IHC confirmation test were performed. The reactivity of established mAb were checked on 5 paraffin embedded bladder cancer tissue in parallel to an approved commercial competitor.

Results: In this study, we successfully produced a stable anti-PD-L1 mAb clone 1D6. It can specifically bind to PD-L1 of normal human placental and tonsil paraffin embedded tissues in IHC. In addition, total agreement was completely observed in comparison study of staining pattern of 1D6 and commercial competitor anti-PD-L1 mAb. In other words, 5 out of 5 bladder cancer tissues were positively stained by both anti-PD-L1 mAbs.

Conclusion: In conclusion, the new developed anti-PD-L1 mAb could be applicable in IHC testing to improve prognosis, diagnosis and treatment outcome of patients with cancer.

Keywords: PD-L1, Immunohistochemistry, Monoclonal antibody, Anti-PD-L1

Production, Purification, and Characterization of Human Heavy Chain Ferritin (Hfn) For Use as a Nanocarrier

Misagh Rajabinejad[1], Ahmad Najafi[1], Mohsen Tehrani[2], Reza Valadan[2], Hossein Asgarian-Omran[2]

1. Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: Introducing precise drug delivery systems for targeted immunotherapy of cancers would significantly improve clinical efficacy and diminish potential side effects. Human heavy chain ferritin (HFn)-based nanocages showed enormous promise in this area, because of their unique structure, self-assembly properties, high biocompatibility, and receptor-mediated endocytosis. This study aimed to produce, purify, and characterize the recombinant HFn nanocarrier in a prokaryotic system for further application as an efficient drug delivery system.

Methods: The cDNA sequence of HFn was cloned into the pET28a expression plasmid. HFn/pET28a plasmid was then transformed into host E. coli BL21 through the CaCl2-mediated transformation process. The transformed BL21 was cultured in LB medium containing chloramphenicol (35 mg/L) and kanamycin (50 mg/L) at 37°C, and the Isopropyl β- d-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM at 27°C overnight when the OD600 reached 0.5. Taking advantage of its C-terminal His-tag, HFn was then purified with Ni-affinity chromatography with a yield of 135 µg/ml. The His-tagged recombinant HFn was eluted with 500 mM imidazole. The eluted fraction of HFn was dialyzed overnight against 10 mM PBS at 4°C. The purity and identity of recombinant HFn were analyzed by 15% SDS-PAGE and immunoblotting, respectively. The concentration of the collected protein was measured using the Bicinchoninic acid (BCA) assay.

Results: Following induction with IPTG at 27°C, a significant expression of HFn was observed compared to the uninduced samples. In SDS-PAGE, a sharp and distinct protein band with a molecular weight of about 20 kDa corresponding to HFn was detected in induced samples. The immunoblotting with anti-histidine antibodies confirmed the presence of soluble HFn in the final product. Under the optimized conditions, which were achieved when the temperature was lowered from 30°C, soluble HFn was produced with a purity of more than 95% and a yield of 135 µg/ml.

Conclusion: The initial results of our study showed that HFn nanocarrier can be produced in a prokaryotic host, providing an effective and economical procedure with good cytoplasmic solubility for scale-up production. We are continuing our studies using HFn nanocarrier as a drug delivery system for treating acute myeloblastic leukemia.

Keywords: Human H-chain ferritin, Nanocarrier, Recombinant protein, Targeted therapy

Scalable Culture Strategy for the Expansion of Natural Killert Cells from Umbilical Cord Blood

Delbar Daneshjou[1], Parisa Shams[2], Marzieh Ebrahimi[3]

1. Kian Immune Cell Co. (KIC), Iran.
2. Department of Cell and Developmental Biology, Faculty of sciences and advanced Technologies in biology, University of science and Culture, Iran.
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Iran.

Background: Natural killer T cells (NKT) cells are a subset of CD1drestricted T cells at the interface between the innate and adaptive immune systems and have characteristics of both conventional T cells and NK cells. Though due to their potential cytotoxicity are noticed by scientists as a candidate for cancer immunotherapy, however, the main challenge is to obtain a sufficient amount of highly pure NKT cells for therapeutic infusion because of the low frequency and number of NKT cells (1-2%) in the peripheral blood. Therefore, several methods have been developed to purify and expand these cells. In this study, we try to develop a simple method for ex vivo expansion and purification of NKT cells from cord blood mononuclear cells (CBMNCs) without feeder cells.

Methods: CBMNCs were harvested from cord blood via Ficoll-Hypaque density gradient centrifugation and were cultured in gas-permeable G-Rex® 6 Well Plate with RPMI1640 medium, 5% autologous serum in the presence of the combination of cytokines for effective expansion of NKT cells for 21 days. On days7, 14 and 21 cell viability, fold increase and NKT purity were analyzed.

Results: Our data shows that the use of G-Rex and cytokines resulted in a 146-fold increase in the number of NKT cells.

Conclusion: This method has strong advantages over existing procedures, as it allows easier, time saving, and cost-effective production of NKT cells.

Keywords: Immunotherapy, Grex, NKT cell, Cord blood

Targeting the Tumor Microenvironment by Liposomal Epacadostat in combination with Liposomal gp100 Vaccine

Sahar Tahaghoghi-Hajghorbani[1], Alireza Rafiei[2], Mona Yazdani[3], Amin Reza Nikpoor[4], Mahdi Hatamipour[5], Abolghasem Ajami[1], Mahmoud Reza Jaafari[6], Ali Badiee[6]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
3. Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Immunology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
5. Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
6. Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Indoleamine-2, 3-dioxygenase (IDO1) pathway has vital role in cancer immune escape and its upregulation leads to immunosuppressive environment which is associated with poor prognosis and progression in various cancers like melanoma. Previously, we showed the antitumoral efficacy of nanoliposomal form of Epacadostat (Lip-EPA), as an IDO1 inhibitor. Herein, we used Lip-EPA as a combination approach with liposomal gp100 (Lip-gp100) anti-cancer vaccine in melanoma model.

Methods: Gp100 vaccine encapsulated in liposomes were formulated using a film method. Their physicochemical properties and encapsulation efficacy were evaluated. B16F10 tumor bearing C57BL6 mice were immunized with different formulation of intravenous therapeutic EPA and/or subcutaneous gp100 vaccine and then were monitored for tumor size and survival throughout the study. Lymphocytes in the spleen, lymph node and tumor infiltrated cells were evaluated using flow cytometric analysis and IFN-γ secretion were measured by Enzyme-linked immunospot (ELISpot) assay.

Results: In an in vivo study, Lip-EPA enhanced the antitumor efficacy of Lip-gp100 in which the IDO mRNA expression was decreased (~ 4-fold) in tumor samples. Also, we identified a significant increase in the number of infiltrated T lymphocytes with enhanced in interferon gamma (IFN-γ) production. Additionally, Lip-EPA+Lip-gp100 significantly modulated intratumoral regulatory T cells which altogether resulted in the highest delay in tumor growth and increased life span in treated mice.

Conclusion: In conclusion, our study demonstrated that novel combination of Lip-EPA and Lip-gp100 was significant on IDO1 inhibition, the immunostimulatory effects, as well as preclinical outcomes, including survival and tumor growth rate and also was effective treatment with capability of being used in further clinical studies.

Keywords: Melanoma, IDO inhibitor, Epacadostat, liposome, Vaccine

The Effect of Engineered-TP53 on Breast Cancer Cells Growth by The Advantage of Adeno-associated Viral Particles Delivery System

Negar Moghare Dehkordi[1], Sadegh Paydari Rostami[1], Mohammad Reza Bolouri[2], Azam Samei[3], Yazdan Asgari[1], Reza Falak[2], Gholam Ali Kardar[1]

1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Clinical Laboratory Sciences, School of Allied Medical Sciences, Kashan University of medical Sciences, Kashan, Iran

Background: Accumulation of mutations in the genome, especially in the tumor suppressor p53 (TP53) sequence can gradually promote normal cells toward cancer development. As a treatment strategy, regeneration of TP53 could induce apoptosis and cell cycle arrest in tumor cells. In this study, by developing, an adeno associated viral vector (AAV) with an engineered TP53 sequence (AAV-TP53), we tried to remove the inhibitory effects of cellular factors on TP53 as well as enhance TP53 homo-tetramerization, and consequently, increase its tumor suppressor effects.

Methods: After optimization of the target residues in the TP53 sequence (vector designation) and production of AAV-TP53 in HEK293T cells, the virus was transduced in SKBR3 breast cancer cells. The expression/activation of engineered TP53 downstream genes, including p21 (as the most critical downstream gene), and BAX (as an apoptosis-related gene) were examined by relative Real-time PCR.

Results: The apoptosis rate was also measured by AnnexinV-PE/ 7AAD staining. The engineered TP53 was highly expressed in SKBR3 cells; consequently, the p21 and BAX genes' expression levels were significantly increased. Furthermore, transduced cells demonstrated a higher apoptotic rate than control and mock samples.

Conclusion: We showed that using engineered TP53 could be an effective method among many TP53-related gene therapy methods for cancer treatment.

Keywords: Tumor suppressor protein p53, Gene therapy, Adeno-associated virus, Apoptosis, Breast cancer.

The producing a medicin food with recombinant lactococcus lactis as a condidate for Immunotherapy

Leila Roozbeh Nasiraie[1]

1. Islamic Azad University, Noor Branch, Iran.

Background: Recently with the increasing prevalence of type I allergy, active delivery of recombinant molecules to mucosal surfaces by genetically lactic acid bacteria is represent a novel and attractive vaccination approach. During the last two decades, significant advances have been made in lactococcal genetics and protein expression systems. Lactococcus lactis is a homofermentative microorganism widely used in the dairy industry as a starter in milk fermentation which recently has increasingly been used to deliver bioactive compounds by different mucosal routes and to treat diseases. It has been considered an ideal candidate for mucosal immunotherapy in allergy treatment.

Methods: Profilin (Che a 2), the major allergen in Chenopodium album is one of the most important causes of allergic diseases in the desert and semi-desert areas, especially in Iran, Saudi Arabia, and Kuwait. Another hand, the viability reduction of Lactococcus lactis in the human gastrointestinal tract is a problem inherent in the use of L. lactis as a delivery vehicle. This study aimed to construct a recombinant Lactococcus lactis ssp. Cremoris MG1363 producing the major chenopodium album pollen allergen (Che a 2), and to determine the survival of free and encapsulated Lactococcus lactis in ice cream during shelf life (at -18ºC for 9o days) and exposure to the adverse conditions of the gastrointestinal tract.To construct L.lactis that expresses Che a 2, the DNA sequence was cloned and transformed into bacteria. Expression of the Che a 2 gene was analyzed via monitoring of related RNA and immunological methods that were applied at protein level. Hydrophobicity, adherence to HT-29 cell line, antibiotic resistance test, resistance to gastrointestinal contents, low pH, and bile salt in recombinant and native Lactococcus lactis were evaluated. Then, the bacteria cells encapsulated into Ca-alginate coated with whey protein capsules by the emulsion technique and inoculated to ice cream (3% fat).

Results: Immunoblot analyses have shown that recombinant Che a 2 is expressed as a dimeric protein with a molecular weight of 32 kDa and is able to bind to human IgE. Both native and recombinant bacteria were sensitive to low pH and simulated gastric conditions. The results showed a %80-100 reduction in bacterial survival after 2h exposure to pH 1.5-2. The result of bile salt tolerance showed both bacteria are able to grow in the presence of 0.3% and 2% bile salt. After incubation of recombinant Lactococcus lactis in simulated gastric juice (60 min) and simulated intestinal juice (2 h), the cell's survival was reduced to %100. Adhesion capability in both strains was at a minimum state and there was no significant difference between native and recombinant bacteria. The ice cream inoculated with free and encapsulated recombinant L.lactis had a final concentration of 1×104 CFU/g and 1.5×107 CFU/g and the survival rate was 28.5% and 50%, respectively, at the end of time storage (90 days). The encapsulating recombinant L.lactis in alginate-whey protein and incorporating it with ice cream both of them caused to surviving %38.5, 37.3%, and 50% under exposure to gastro-intestinal conditions, respectively, compared to free cells that were %0.

Conclusion: In general, the results indicated that microencapsulation and incorporation with ice cream as a suitable carrier can significantly protect the L.lactis against gastrointestinal adverse conditions. The addition of encapsulated probiotics had no significant effect on the sensory properties of non-fermented ice cream.

Keywords: Che a 2, Ice creMicroencapsulation, Recombinant Lactococcus lactis, Simulated gastrointestinal conditions

Mucosal Immunology

Ameliorative effects of silver nanoparticles synthesized using the aqueous extract of Artemisia Annua on ulcerative colitis in rats

Seyyed Jamaleddin Tabatabaei Aghda[1], Mohammad Mahdi Zangeneh[2], Navid Etemadi[3], Akram Zangeneh[2]

1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University
2. Biotechnology and medicinal plants Research center, Ilam University of medical sciences
3. DVM, Faculty of veterinary Medicine, Urmia University

Background: Nanotechnology has improved medical science both in the diagnosis and monitoring of disease and in the treatment. The aim of this study was to investigate the antioxidant activities and protective effect of Silver nanoparticles against ulcerative colitis in rats.

Methods: In this study, 40 adult male rats were divided into 5 groups of 8, randomly: Healthy control group, Patient control group, Patient group receiving Prednisolone (60mg/kg), Patient group receiving Artemisia Annua aqueous extract (200mg/kg), patient group receiving silver nanoparticles of Artemisia aqueous extract (0.5mg/kg). To induce ulcerative colitis, acetic acid was injected intra_rectally. After confirming the induction of the disease, treatment period was started in rats. After ten days, plasma and serum of rats were sent for hematological and biochemical tests and intestines of rats for histopathology. The obtained results were fed into SPSS-22 software and analyzed by one-way ANOVA. These nanoparticles were characterized by FT-IR, UV, XRD, FE-SEM, TEM, and AFM.

Results: The results showed that the level of AST, ALT, ALP, GGT, cholesterol, LDL, triglyceride, Creatinine, HDL, total protein, WBC, hemoglobin, RBC, PCV, MCV, MCH and MPO, MDA, NO and also necrosis, erythrocyte congestion and accumulation of inflammatory cells in the intestinal tract were significantly reduced in the group receiving silver nanoparticles compared to other groups. On the other hand, macroscopic examination of tissues showed a significant decrease in the level of all three types of small, large and medium wounds in this group compared to other groups.

Conclusion: These results demonstrated the silver nanoparticles (AgNPs) of Artemisia Annua aqueous extract as Suitable chemical composition is a promising strategy to improve the inflammation in a rat model of ulcerative colitis.

Keywords: Silver nanoparticles (AgNPs), Artemisia Annua aqueous extract, ulcerative colitis, acetic acid

Anti-histaminic Effects of Resveratrol and Silymarin on Human Gingival Fibroblasts

Minoo Shahidi[1]

1. Department of Hematology & Blood Banking, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran

Background and objectives: Periodontitis as a chronic inflammatory disease leads to the destruction of the supportive tissues of affected teeth. Crosstalk between periodontitis and the host immune system plays a crucial role in the pathogenesis of this disease. Since polyphenol components such as silymarin and resveratrol have anti-bacterial and anti-inflammatory effects on periodontal tissues, the purpose of this study was to investigate the anti-histaminic effects of silymarin and resveratrol on human gingival fibroblasts (HGFs).

Methods: HGFs were treated with a concentration of silymarin or resveratrol (100 µg/ml) and a combination of these two polyphenols (50/100 or 100/200 µg/ml silymarin/resveratrol). The effect of silymarin and resveratrol on cell viability was assessed by MTT assay. Also, HGFs were treated with silymarin and/or resveratrol and were stimulated by histamine. The levels of interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), and tissue plasminogen activator 1 (TPA-1) were assessed by enzyme-linked immunosorbent assay (ELISA).

Results: After treatment with silymarin, the viability of fibroblast cells significantly increased, whereas treatment with resveratrol and combinations of these flavonoids (silymarin 50 µg/ml and resveratrol 100 µg/ml) did not have any significant effect on cell viability after 24 h. Treatment with 100/200 µg/ml silymarin/resveratrol significantly decreased the cell viability after 48 h. Resveratrol inhibited histamine-induced IL-6 secretion by HGFs significantly, whereas silymarin showed a significant effect on TNF-α A blend of silymarin and resveratrol displayed more valuable results.

Conclusion: Taking together, a combination of resveratrol and silymarin could significantly inhibit the inflammatory effects of histamine on cultured HGFs by reduction of IL-6, IL-8, TPA-1, and TNF-α

Keywords: Histamine, resveratrol, silymarin, inflammatory effects

Anti-inflammatory Activity of nanoparticles synthesized with Falcaria vulgaris (paghazeh) on gastric ulcer in rats

Maryam Almasi[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[2]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
2. Biotechnology and medicinal plants research center, Ilam University of medical sciences, Ilam, Iran

Background: Gastric ulcer is one of the most common chronic gastrointestinal diseases characterized by a notable defect in the mucosal barrier. Falcaria vulgaris is a species of pasture plants, an annual of the family of Apiaceae, and is effective in the treatment of digestive inflammations and stomach ulcers with its protective efficacies. The purpose of this study is to investigate the effect of Falcaria vulgaris extract and nanoparticles synthesized from it in the healing of gastric ulcer.

Methods: 48 rats were placed in 6 groups and gavage was given for 12 days: Control group (physiological serum), patient control group, patient group receiving omeprazole 60 mg/kg, Patient group gavage with F.vulgaris extract (200 mg/kg), Patient group gavage with silver F.vulgaris nanoparticle (0.5 mg/kg), and Patient group gavage with copper F.vulgaris nanoparticle (0.5 mg/kg). After 12 days, 0.5 cc of ethanol was given to the rats and then dissected, Blood and stomach samples were taken. Histopathology studies and AST, ALT, ALP, GGT, triglyceride, cholesterol, LDL, HDL, urea, total protein, creatinine, WBC, RBC, Hb, HGB, PCV, MCV, MCH factors were performed. The results were analyzed using Tukey's test in SPSS26 software (p<0.05).

Results: Biochemical factors were significantly reduced in nanoparticle groups compared to other groups. At the level of the stereoscope, the number of small, medium and large wounds decreased in the groups that received nanoparticles. In the pathological observations, in the nanoparticle groups, the accumulation of inflammatory cells was significantly reduced compared to other groups. MDA, NO, MDA markers were also remarkably reduced in the F.vulgaris nanoparticle receiving groups.

Conclusion: Falcaria vulgaris with its anti-inflammatory and antibacterial properties and improving the function of liver enzymes and blood factors, it prevents the development of stomach ulcers.

Keywords: Falcaria vulgaris, gastric ulcer, copper nanoparticles, silver nanoparticle

Anti-inflammatory effect of silver and copper nanoparticles of Allium Saralicum on gastric ulcer in mice

Mohammadmahdi Zangeneh[1], Samira Zand[2], Akram Zangeneh[1]

1. Biotechnology and Medicinal plants Research Center Ilam University of Medical Sciences, Ilam, Iran
2. Department of Microbiology, Faculty of veterinary Medicine, Urmia University, Urmia, Iran

Background: Gastric ulcer is a common disorder of the digestive system. Several studies have reported that herbal medicines can effectively treat gastric ulcers and display fewer adverse effects. Gold nanoparticles have antioxidant behaviors including superoxidase dismutase and nitric oxide. Hence, we decided to design a study to determine the anti-inflammatory effects of nanoparticles of Allium Saralicum on gastric ulcers in mice.

Methods: Forty male mice were divided into 5 groups containing 8 which were gavaged for 12 days. The negative healthy group was given only physiological saline, the positive control group received omeprazole (60 mg/kg). Two treatment groups received Allium Saralicum extract (200 mg/kg) and silver/copper Allium Saralicum nanoparticle (0.5 mg/kg). After 12 days, 0.5 ccs of ethanol were given to the mice and then dissected.

Results: Our findings showed that the silver nanoparticles of Allium Saralicum were able to notably reduce the levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total bilirubin, urea, and creatinine and also improve the levels of HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the other treatment groups. Further, the silver nanoparticles of Allium Saralicum significantly reduced the rate of liver necrosis, erythrocyte congestion, and inflammation of inflammatory cells compared to the other groups. It also reduces NO, MPO, and MDA levels in plasma compared to other treatment groups.

Conclusion: copper and silver nanoparticles of Allium Saralicum have anti-inflammatory effects on gastric ulcers in mice.

Keywords: Gastric ulcer, Allium Saralicum, silver nanoparticles, copper nanoparticles

Association of the Reduced Level of Interleukin-13 in Breast Milk with Chronic Diarrhea in Infancy

Sepideh Moradkhani[1], Mohammad Mahdi Mohammadi[1], Fatemeh Minoochehr[2], Matin Vesaltalab[3], Seddighe Amini-Ranjbar[4], Mohammad Reza Baneshi[5], Farhad Seif[6], Majid Khoshmirsafa[6]

1. Department of Immunology, School of Medicine, University of Kerman
2. Department of Human Genetics Research Center, Faculty of Medicine, University of Baqiyatallah
3. Faculty of Medicine, University of Bandar abbas, Iran.
4. Department of Gastroenterology, Faculty of Medicine, University of Kerman
5. Department of Modeling in Health Research Center, School of Medicine, University of Kerman
6. Department of Immunology, School of Medicine, University of Iran

Background: Chronic diarrhea (CD) is one of the major diseases during infancy. The effects of various substances in breast milk on preventing chronic diarrhea are not fully elucidated. This study aimed to determine the concentration of inflammatory cytokines of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), and anti-inflammatory cytokines of interleukin-4 (IL-4) and interleukin-13 (IL-13) through the mothers' breast milk feeding the infants with and without CD.

Methods: Breast milk samples were obtained from 45 mothers feeding the infants with CD as the case group and 45 mothers feeding healthy infants without CD (or any other inflammatory diseases) as the control group. The concentration of inflammatory and anti-inflammatory cytokines in breast milk was measured using ELISA.

Results: The mean of IL-13 concentration was significantly reduced in the case group compared to the control group (p<0.001). Whereas the mean of IL-4 concentration was significantly increased in the CD group (p=0.001).

Conclusion: The results indicated a lower IL-13 concentration and a higher IL-4 concentration in the mothers feeding the infants with CD. Therefore, low IL-13 as an anti-inflammatory cytokine in breast milk may be capable of predisposing infants to CD. On the other hand, inflammatory cytokines may promote the immunity of infants with CD.

Keywords: breastfeeding, chronic diarrhea, cytokines, infant

Cell Density Counts of the Intestinal Intraepithelial Lymphocytes in the Celiac Patients

Mohsen Masjedi[1], Hadi Hossein Nataj[2], Sayyed Mohammad Hassan Emami[1], Mojgan Mokhtari[1], Fereshteh Alsahebfosoul[1]

1. Isfahan University of Medical Sciences, Isfahan, Iran.
2. Mazandaran University of Medical Sciences, Sari, Iran.

Background: Increased number of intestinal intraepithelial lymphocytes (IELs) is a key histological finding in the diagnosis of celiac disease (CD); however, the number of IELs in celiac patients and healthy subjects may vary from one region to another. Additionally, there are some seronegative celiac patients with borderline histology.

Objective: To determine the number of CD3+ and CD8+ IELs T-cells in the celiac patients and healthy subjects (controls) in Isfahan. Methods: The duodenal biopsies were obtained from the celiac patients (n=15) and the controls (n=19). The total number of IELs/100 epithelial cells (ECs) were counted using the hematoxylin-eosin (H&E) staining method, and that of CD3+ and CD8+ IELs/100 ECs were counted using the immunohistochemistry (IHC) staining method.

Results: This study defined the upper normal limit for each variable as mean + 2SD. Accordingly, the upper normal limits of the total IELs, CD3+ IELs, and CD8+ IELs/100 ECs were calculated as 37 (95% confidence intervals, CI: 33–41), 22 (95% CI: 19–25) and 12 (95% CI: 10–14), respectively. In 3 clinical CD diagnoses, the total IELs counts/100 ECs were below the upper normal limit, and the histopathological and serologic assays were negative. Nevertheless, the CD8+ IELs T-cell counts/100 ECs showed borderline values. Interestingly, these patients responded to a gluten-free diet (GFD).

Conclusion: The study findings suggest that in the clinically diagnosed celiac disease, IELs count/100 ECs below the upper normal limit as well as negative histopathological and serologic assays and the cell density counts of the CD8+ IELs T-cells/100 ECs could be a useful parameter for CD diagnosis and make a decision to put them on a GFD.

Keywords: "Celiac Disease", "Diagnosis", "Gut", "Intraepithelial Lymphocytes".

Evaluation of Inhibitory Effect of Abatacept (CTLA4-ig) and Conditioned Medium of Mesenchymal Stem Cell in an Acetic Acid-induced Mouse Model of Acute Colitis

Manizhe Faghih[1], Mona Moshiri[1], Fatemeh Ahmadzadeh[1], Maryam Ghasemi[1], Saeed Abediankenari[1]

1. Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. Immunogenetics Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

Background: A genetically predisposed individual is affected by inflammatory bowel disease (IBD), which causes inflammatory responses that led to Crohn's disease (CD) or Ulcerative colitis (UC). It is currently being emphasized that stem cell therapies, especially those using mesenchymal stem cells (MSCs), have immunomodulatory properties because they have proven effective in clinical trials. So, we examined the effects of mesenchymal stem cells-conditioned medium (MSC-CM) and Abatacept in the experimental model of acute colitis.

Methods: MSC-CM was isolated from BALB/C female mice and stored. The acute colitis induction in BALB/C mice was performed by intrarectal injection of 4% acetic acid at a dose of 100 µL and then CM and Abatacept were injected intraperitoneally. During the study body weight changes, bleeding, stool consistency, disease activity index (DAI), mortality rate, weight and length of the colon and histopathological analysis were recorded as well as changes in the level of IL-10 and IFN-γ. Data are reported as mean ± SD and analyzed using One-Way ANOVA.

Results: According to the results, CM with and without an Abatacept significantly reduced weight loss and bleeding as well as improved fecal consistency and DAI. Macroscopic examination of the colon showed that after infusion, colon inflammation was reduced and histopathological analysis showed a decrease in mucosal changes. The secretion of IL-10 was increased while the IFN-γlevel was reduced.

Conclusion: Studies have shown that mesenchymal stem cell secretion with immunomodulatory properties has beneficial effects.

Keywords: Colitis, Acetic Acid, Abatacept, Mesenchymal Stem Cell, Conditioned Medium

Investigating the effect of silver and zinc nanoparticles on the aqueous extract of Matricaria chamomilla plant on ulcerative colitis in male rats

Navid Etemadi[1], Akram Zangeneh[2], Mohammad Mehdi Zangeneh[2], Naser Abbasi[2]

1. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.
2. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.

Background: The use of medicinal plant material increases the therapeutical effects of zinc and silver nanoparticles.

Methods: These nanoparticles were characterized by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), ultraviolet–visible spectroscopy (UV), transmission electron microscopy (TEM), and field emission scanning electron microscopy (FE-SEM) analysis. 48 adult male rats were divided into 6 groups of 8, randomly: Healthy control group, the Patient control group, the Patient group receiving Prednisolone (60mg/kg), Patient group receiving aqueous Matricaria chamomilla extract (200mg/kg), Patient group receiving silver nanoparticles of Matricaria chamomilla aqueous extract (0.5mg/kg) and Patient group receiving zinc nanoparticles of Matricaria chamomilla aqueous extract (0.5mg/kg). To induce ulcerative colitis, acetic acid was injected intra_rectally. After confirming the induction of the disease, the treatment period was started in rats. After ten days, plasma and serum of rats were sent for hematological and biochemical tests and the intestines of rats for histopathology.

Results: The results showed that macroscopic examination of tissues showed a significant decrease in the level of all three types of small, large and medium wounds in the group receiving silver nanoparticles compared to other groups. the level of AST, ALT, ALP, GGT, cholesterol, LDL, triglyceride, Creatinine, HDL, total protein, WBC, hemoglobin, RBC, PCV, MCV, MCH and MPO, MDA, NO and also necrosis, erythrocyte congestion and accumulation of inflammatory cells in the intestinal tract were significantly reduced in this group.

Conclusion: The results of XRD, FT-IR, UV, TEM, and FE-SEM confirm that the silver nanoparticles on the aqueous extract of Matricaria chamomilla may be a promising agent to ameliorate ulcerative colitis.

Keywords: silver nanoparticles; Matricaria chamomilla; ulcerative colitis

Investigating the effect of sodium butyrate on TLR4 gene expression pattern and NF-KB p65 expression in colorectal cancer cell lines

Nazanin-Atieh Kazemi-Sefat[1], Hamid Daneshvar[2], Zuhair Mohammad Hassan[1]

1. Department of Medical Immunology, Faculty of Medical Sciences, Tarbiat Modares University (TMU), Tehran, Iran.
2. Kerman Physiology Research Center (KPRC), Kerman University of Medical sciences (KMU), Kerman, Iran.

Background: Toll-like receptor-4 (TLR4) is important as an innate immune receptor in mucosal epithelial cells and colorectal cancer (CRC). NF-KB p65 is one of the NF-KB subunits which is a pivotal marker in the targeted therapy approach. Our main investigation was focused on the effect of sodium butyrate (SB) generated by gut microbiota as an HDAC inhibitor on TLR4 expression levels and NF-KB p65 in the TLR4 signaling, in HCT116 and SW480 CRC cell lines with different baseline TLR4 expression.

Methods: The cytotoxicity of SB was determined by MTT assay. In addition, the apoptosis test was done using flow cytometry. The relative gene expression of TLR4 at the RNA level was evaluated by Real Time PCR. Using the Western blotting method, NF-KB p65 expression was determined at 1mM of SB for HCT116 and 5mM of SB for SW480.

Results: Morphological analysis, MTT assay and apoptosis test showed that 5mM or less SB concentrations were not cytotoxic. At 1mM of SB in HCT116 as well as 5mM of SB in SW480, TLR4 gene expression level significantly elevated from 24 to 48 hrs and diminished significantly from 48 to 72 hrs with an "early increased and late decreased pattern". At 5mM of SB in HCT116 along with 1mM of SB in SW480, TLR4 expression had a "gradually increased pattern". NF-KB p65 was diminished after 72h at 1mM and 5mM of SB, in HCT116 and SW480 cell lines, respectively.

Conclusion: Our data, has shown special patterns of TLR4 gene expression following SB treatment. These patterns may depend on the cell type, treatment duration and SB concentration. The alterations in TLR4 expression may be due to the direct effect of SB on TLR4 and/or the expression changes of other genes which may indirectly affect the TLR4 expression. SB effect on TLR4 expression may regulate intestinal homeostasis and innate immunity in CRC.

Keywords: TLR4, sodium butyrate, colorectal cancer

The gold nanoparticles synthesized using the aqueous extract of Artemisia dracunculus effectively prevent induced gastroduodenal ulcer

Seyyed Jamaleddin Tabatabaei Aghda[1], Mohammad Mahdi Zangeneh[2], Navid Etemadi[3], Akram Zangeneh[3]

1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University.
2. Biotechnology and medicinal plants Research center, Ilam University of medical sciences
3. DVM, Faculty of veterinary Medicine, Urmia University

Background: Because many people in the world suffer from gastroduodenal ulcers, therefore, studying the therapeutic strategies of these ulcers are research priority in any country. The aim of the new study was to survey the preventive property of gold nanoparticles (AuNPs) of Artemisia dracunculus on ethanol-induced gastroduodenal ulcers in rats.

Methods: In this study, 35 rats were used. The rodents were randomly divided into five subgroups, including negative healthy control receiving distilled water, untreated negative control receiving distilled water, positive control receiving omeprazole 60 mg/kg, one group receiving the aqueous extract of Artemisia dracunculus at 200 mg/kg concentrations, and a group receiving the of gold nanoparticles (AuNPs) of aqueous extract Artemisia dracunculus at 5 mg/kg concentrations. After 14 days, gastroduodenal ulcers were caused by ethanol. Four hours after oral administration of ethanol, the rats were dissected and blood, stomach, and duodenum samples of them collected for hematological, biochemical, Immunology, and gross parameters analysis. The data were analyzed using one-way ANOVA followed by Duncan post hoc test.

Results: The gold nanoparticles (AuNPs) of aqueous extract Artemisia dracunculus could significantly ($p\leq 0.05$) decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the other groups. Also, the gold nanoparticles (AuNPs) of the aqueous extract Artemisia dracunculus prevented significantly ($p\leq 0.05$) small, medium and large gastroduodenal ulcers as compared to the other groups. It also further reduces NO, MPO and MDA compared to other groups.

Conclusion: It seems that the gold nanoparticles (AuNPs) of the aqueous extract Artemisia dracunculus can prevent gastroduodenal ulcers in rats without any side effects.

Keywords: gold nanoparticles (AuNPs) of aqueous extract Artemisia dracunculus, gastroduodenal protective, Ethanol

The gold and silver nanoparticles synthesized using the aqueous extract of Artemisia Dracunculus effectively prevent induced gastroduodenal ulcer.

Navid Etemadi[1], Akram Zangeneh[2], Mohammad Mehdi Zangeneh[2], Naser Abbasi[2]

1. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.
2. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.

Background: The aim of the new study was to survey the preventive property of gold and silver nanoparticles of Artemisia Dracunculus on ethanol-induced gastroduodenal ulcers in rats.

Methods: In this study, 42 rats were used. The rodents were randomly divided into six subgroups, including negative healthy control receiving distilled water, untreated negative control receiving distilled water, positive control receiving omeprazole 60 mg/kg, one group receiving the aqueous extract of Artemisia Dracunculus at 200 mg/kg concentrations, a group receiving the of silver nanoparticles (AgNPs) of aqueous extract Artemisia Dracunculus and a group receiving the of gold nanoparticles (AuNPs) of aqueous extract Artemisia Dracunculus at 5 mg/kg concentrations. After 14 days, gastroduodenal ulcers were caused by ethanol. Four hours after oral administration of ethanol, the rats were dissected and blood, stomach, and duodenum samples of them collected for hematological, biochemical, Immunology, and gross parameters analysis.

Results: The gold nanoparticles (AuNPs) of aqueous extract Artemisia Dracunculus could significantly ($p\leq 0.05$) decrease the raised levels of ALP, ALT, GGT, cholesterol, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCH, and MCHC as compared to the other groups. Also, the gold nanoparticles (AuNPs) of the aqueous extract Artemisia Dracunculus prevented significantly ($p\leq 0.05$) small, medium and large gastroduodenal ulcers as compared to the other groups. It also further reduces NO, MPO and MDA compared to other groups.

Conclusion: It seems that the gold nanoparticles (AuNPs) of aqueous extract Artemisia Dracunculus can prevent gastroduodenal ulcers in rats without any side effects.

Keywords: gold nanoparticles (AuNPs) of aqueous extract Artemisia Dracunculus; gastroduodenal protective; ethanol.

The silver and zinc nanoparticles synthesized using the aqueous extract of Matricaria chamomilla effectively prevent induced ulcerative colitis.

Akram zangeneh[1], Mohammad Mehdi zangeneh[2], Neda Ahmadiafshar[3], Mojtaba Moradi[1], Somayeh Ahmadiafshar[1]

1. Urmia University of medical sciences, Ilam, Iran.
2. Ilam University of medical sciences, Ilam, Iran.
3. Tehran University of medical sciences, Ilam, Iran.

Background: The new ground being broken by the field of nanotechnology provides us with numerous prospects for the treatment and prevention of diseases. The aim of this study was to investigate the antioxidant activities and protective effect of silver and zinc nanoparticles against ulcerative colitis in rats.

Methods: In this study, 40 adult male rats were divided into 5 groups, randomly: Healthy control group, Patient control group, Patient group receiving Prednisolone (60 mg/kg), Patient group receiving Matricaria chamomilla aqueous extract (200mg/kg), Patient group receiving silver and zinc nanoparticles of Matricaria chamomilla aqueous extract (0/5 mg/kg).To induce ulcerative colitis, acetic acid was injected intra_rectally. After confirming the induction of the disease, the treatment period was started in rats. After ten days, the plasma and serum of rats were sent for hematological and biochemical tests and the intestines of rats for histopathology.

Results: The results showed that the level of AST, ALT, ALP, GGT, cholesterol, LDL, triglyceride, Creatinine, HDL, total protein, WBC, hemoglobin, RBC, PCV, MCV, MCH and MPO, MDA, NO and also necrosis, erythrocyte congestion and accumulation of inflammatory cells in the intestinal tract were significantly reduced in the group receiving nanoparticles compared to other groups. On the other hand, macroscopic examination of tissues showed a significant decrease in the level of all three types of small, large and medium wounds in this group compared to other groups.

Conclusion: This study showed that the use of silver and zinc nanoparticles containing Matricaria chamomilla extracts has significant antioxidant and protective effects compared to the use of this plant alone or the use of prednisolone in the treatment of ulcerative colitis in rats.

Keywords: ulcerative colitis, silver and zinc nanoparticles, Matricaria chamomilla, Rat

The zinc and gold nanoparticles synthesized using the aqueous extract of Falcaria vulgaris effectively prevent induced ulcerative colitis.

Somayeh Ahmadiafshar[1], Ali Pirnejad[1], Neda Ahmadiafshar[2], Mojtaba Moradi[3], Mohammad Mehdi Zangeneh[3], Akram Zangeneh[1]

1. Urmia University of medical sciences, Ilam, Iran
2. Tehran University of medical sciences, Ilam, Iran.
3. Ilam University of medical sciences, Ilam, Iran.

Background: The exciting advances within nanotechnology are beginning to be harnessed by the medical field. Nanoparticles have been used for drug delivery in medicine. The aim of this study was to investigate the antioxidant activities and protective effect of zinc and gold nanoparticles against ulcerative colitis in rats.

Methods: In this study, 40 adult male rats were divided into 5 groups, randomly: Healthy control group, Patient control group, Patient group receiving Prednisolone (60 mg/kg), Patient group receiving Falcaria vulgaris aqueous extract (200mg/kg), Patient group receiving zinc and gold nanoparticles of Falcaria vulgaris aqueous extract (0/5 mg/kg).To induce ulcerative colitis, acetic acid was injected intra_rectally. After confirming the induction of the disease, the treatment period was started in rats. After ten days, the plasma and serum of rats were sent for hematological and biochemical tests and intestines of rats for histopathology.

Results: The results showed that the level of AST, ALT, ALP, GGT, cholesterol, LDL, triglyceride, Creatinine, HDL, total protein, WBC, hemoglobin, RBC, PCV, MCV, MCH and MPO, MDA, NO and also necrosis, erythrocyte congestion and accumulation of inflammatory cells in the intestinal tract were significantly reduced in the group receiving nanoparticles compared to other groups. On the other hand, macroscopic examination of tissues showed a significant decrease in the level of all three types of small, large and medium wounds in this group compared to other groups.

Conclusion: This study showed that the use of zinc and gold nanoparticles containing Falcaria vulgaris extracts has significant antioxidant and protective effects compared to the use of this plant alone or the use of prednisolone in the treatment of ulcerative colitis in rats.

Keywords: zinc and gold nanoparticles, Falcaria vulgaris, ulcerative colitis, Rat

Nanoimmunology

Alleviative Effects of Silver Nanoparticles of the Aqueous Extract Matricaria Chamomilla against Acetaminophen-induced Hepatotoxicity in Mouse

Naser Abbasi[1], Siavosh Kaki Sahneh[2], Navid Etemadi[3], Akram Zangeneh[4], Mohammad Mehdi Zangeneh[1]

1. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.
2. DVM, faculty of veterinary medicine, Urmia University, Urmia, Iran.
3. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.

Background: Metallic nanoparticles have gained significant attention in the area of biomedical technology. Acetaminophen is used to treat mild to moderate fever and pain at a standard dose. However, higher doses may lead to toxicity, including liver failure.

Methods: FeNPs were characterized and analyzed using common nanotechnology techniques including FT-IR, UV–Vis. spectroscopy; EDS, TEM, and FE-SEM.In this study, 56 adult male mice were divided into seven groups. In group 1 as the control group, Physiological serum was given for 30 days. In group 2, acetaminophen (500 mg/kg) was given on day 29. In group 3, an aqueous extract of Matricaria chamomilla (200 mg/kg) was given for 30 days. In group 4, acetaminophen was given according to group 2, and an aqueous extract of Matricaria chamomilla was given according to group 3. In group 5, silver nanoparticles of (200 mg/kg) were given for 30 days. In group 6, acetaminophen was given according to group 2, and silver nanoparticles of Matricaria chamomilla were given according to group 5. Finally, the animal was dissected, and blood and liver samples were collected to evaluate biochemical, immunological, hematological, and Histopathological parameters.

Results: The results indicated that the silver nanoparticles of Matricaria chamomilla were able to notably decrease the levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total bilirubin, urea, and creatinine and also increase the levels of HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC compared to the other treatment groups. Besides, the silver nanoparticles of Matricaria chamomilla considerably diminished liver necrosis, erythrocyte congestion, and inflammation compared to the other groups. Also, it decreased NO, MPO, and MDA levels in plasma compared to other treatment groups.

Conclusion: silver nanoparticles of Matricaria chamomilla prevent the liver against Acetaminophen-induced hepatotoxicity in mice.

Keywords: silver nanoparticles (AgNPs) of aqueous extract Matricaria chamomilla, Hepatotoxicity, Acetaminophen.

Allium saralicum green-mediated silver nanoparticles: formulation, characterization and assessment of colorectal cancer activities

Seyyed jamaleddin Tabatabaei aghda[1], Mohammad Mahdi Zangeneh[2], Navid Etemadi[3], Akram Zangeneh[2]

1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University.
2. Biotechnology and medicinal plants Research center, Ilam University of medical sciences
3. DVM, Faculty of veterinary Medicine, Urmia University

Background: The biosynthesis of metal nanoparticles using medicinal plants is not only economical but also environmentally friendly as well as having miscellaneous biomedical applications.

Methods: The anti-human colorectal cancer activities of AgNPs, A. saralicum were evaluated using MTT assay. The nanoparticles were formed in a spherical shape in the range of 34.24 to 73.12 nm for the particle size. On the other hand, the MTT assay was run to evaluate anti colorectal cancer activity of AgNPs, A. saralicum. In the cellular and molecular part of the study, the cells treated with AgNPs, A. saralicum were assessed by MTT assay for 46 h to determine the cytotoxicity and anti-human colorectal carcinoma properties on normal (HUVEC) and colorectal carcinoma cell lines, i.e., WiDr, SW1417 [SW-1417], and DLD-1. In the present study, gold nanoparticles were green-synthesized using an aqueous extract of Calendula officinalis. The synthesized AgNPs, A. saralicum was characterized by analytical techniques including EDX, FE-SEM, XRD, UV-Vis., and FT-IR.

Results: The IC50 values of AgNPs, A. saralicum were 412, 312, and 370 µg/ml against WiDr, SW1417 [SW-1417], and DLD-1 cell lines, respectively. In the antioxidant test, the IC50 values of AgNPs, A. saralicum and BHT against DPPH free radicals were 228 and 112 µg/ml, respectively. The viability of the malignant colorectal cell line decreased dose-dependently in the presence of AgNPs, A. saralicum.

Conclusion: After the clinical study, silver nanoparticles containing A. saralicum leaf aqueous extract may be used to formulate a new chemotherapeutic drug or supplement to treat several types of human colorectal carcinoma.

Keywords: Allium saralicum green-mediated silver nanoparticles, chemotherapeutic, colorectal cancer activities

Ameliorative effects of gold nanoparticle synthesized using Matricaria chamomilla extract on ulcerative colitis in rats

Siavosh Kaki Sahneh[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[2], Navid Etemadi[3]

1. DVM, faculty of veterinary medicine, Urmia University, Urmia, Iran.
2. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.
3. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.

Background: Nanotechnology has improved medical science both in the diagnosis and monitoring of disease and in treatment. The aim of this study was to investigate the antioxidant activities and protective effect of gold nanoparticles against ulcerative colitis in rats.

Methods: These nanoparticles were characterized by fourier transformed infrared spectroscopy (FT-IR), field emission scanning electron microscopy (FE-SEM), energy dispersive X-ray spectroscopy (EDS), and UV–visible spectroscopy. In this study, 40 adult male rats were divided into 5 groups of 8, randomly: Healthy control group, Patient control group, Patient group receiving Prednisolone (60mg/kg), Patient group receiving aqueous Matricaria chamomilla extract (200mg/kg), and Patient group receiving gold nanoparticles of Matricaria chamomilla aqueous extract (0.5mg/kg). To induce ulcerative colitis, acetic acid was injected intra_rectally. After confirming the induction of the disease, treatment period was started in rats. After ten days, plasma and serum of rats were sent for hematological and biochemical tests and intestines of rats for histopathology.

Results: The results showed that the level of AST, ALT, ALP, GGT, cholesterol, LDL, triglyceride, Creatinine, HDL, total protein, WBC, hemoglobin, RBC, PCV, MCV, MCH and MPO, MDA, NO and also necrosis, erythrocyte congestion and accumulation of inflammatory cells in the intestinal tract were significantly reduced in the group receiving nanoparticles compared to other groups. On the other hand, macroscopic examination of tissues showed a significant decrease in the level of all three types of small, large and medium wounds in this group compared to other groups.

Conclusion: This study showed that the use of gold Matricaria chamomilla nanoparticles containing extracts has significant antioxidant and protective effects compared to the use of this plant alone or the use of prednisolone in the treatment of ulcerative colitis in rats.

Keywords: gold nanoparticles (AuNPs) of aqueous extract Matricaria chamomilla; ulcerative colitis; acetic acid.

Ameliorative effects of nanoparticles synthesized by Artemisia annua on hepatotoxicity induced in mice

Maryam Almasi[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[2], Naser Abbasi[2], Navid Etemadi[3]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran
2. Biotechnology and medicinal plants research center, Ilam University of Medical Sciences, Ilam, Iran
3. DVM Graduated, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Background: Artemisia annua, also known as sweet wormwood, is a common type of wormwood that is native to temperate Asia but occurs worldwide and is a member of the Asteraceae family. In the following study, the effects of A.annua -synthesized nanoparticles, which is a safe method for the environment, on the improvement of liver function during hepatotoxicity have been investigated.

Methods: 48 adult male mice were divided into six groups: 1. control group (Physiological serum for 30 days), 2. Acetaminophen (500 mg/kg were given on day 29), 3. Aqueous extract of A.annua (200 mg/kg were given for 30 days). 4. Acetaminophen with A.annua aqueous extract: Acetaminophen was given according to group 2, and aqueous extract of A.annua were given according to group 3, 5. copper nanoparticles of A.annua (200 mg/kg) were given for 30 days,6: acetaminophen with nanoparticle: acetaminophen was given according to group 2, and cooper nanoparticles of A.annua were given according to group 5. After 30 days, the animal was dissected, and blood and liver samples were collected to appraise hematological, biochemical, immunological, and histopathological factors.

Results: The results revealed that the cooper nanoparticles of A.annua were able to notably reduce the levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total bilirubin, urea, and creatinine and also briefly improve the levels of HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the other treatment groups. Further, the cooper nanoparticles of A.annua significantly reduced the rate of liver necrosis, erythrocyte congestion, and inflammation of inflammatory cells compared to the other groups. Decreased NO, MPO, and MDA levels in plasma was evident compared to other treatment groups.

Conclusion: It could be concluded from the current study that the A.annua nanoparticles can overcome the development of hepatotoxicity.

Keywords: copper nanoparticles, Artemisia annua, hepatotoxicity, Acetaminophen

Anticancer effects of chitosan nanoparticles mediated delivery of miR-340 in breast cancer

Sarvenaz kashefi[1], Mostafa Hajimollahoseini[2]

1. Shahid beheshti medical university, Tehran, Iran.

Background: MicroRNAs (miRNAs) have a crucial role in the regulation of gene expression in tumor development, invasion, and metastasis. Herein, miR-340 has been shown to play tumor suppressor activity in breast cancer. However, the clinical applications of miRNAs request the development of safe and effective delivery systems capable of protecting nucleic acids from degradation.

Methods: In this study, biodegradable chitosan nanoparticles incorporating miR-340 plasmid DNA (pDNA) (miR-340 CNPs) were synthesized and characterized. Then, the antitumor effects of miR-340 CNPs were investigated using 4T1 breast cancer cells. The spherical NPs with an appropriate mean particle size of around 266 nm and zeta potential of +17 mV were successfully prepared. The nanoparticles showed good stability, high entrapment efficiency and a reasonable release behavior, meanwhile their high resistance against enzymatic degradation was verified. Furthermore, nanoparticles demonstrated appropriate transfection efficiency and could induce apoptosis, so had toxicity in 4T1 breast cancer cells. Also, CD47 expression on the surface of cancer cells was significantly reduced after treatment with miR-340 CNPs.

Results: The results showed that miR-340 CNPs augmented the expression of p27 in breast cancer cells. Further, miR-340 CNPs caused down-regulation of BRP-39 (breast regression protein-39) increasingly suggested as a prognostic biomarker for neoplastic diseases like breast cancer.

Conclusion: In conclusion, our data show that miR-340 CNPs can be considered as a promising new platform for breast cancer gene therapy.

Keywords: chitosan nanoparticle, miR340, breast cancer

Anti-inflammasome-based treatment of atherosclerosis using a biomimetic cell membrane-coated nanoparticle

Zahra Karami[1], Mohammad Akrami[2], Jalil Mehrzad[3]

1. Department of Immunology, Faculty of Veterinary Medicine, University of Tehran
2. Department of Pharmaceutical Biomaterials and Medical Biomaterials Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, and Institute of Biomaterials, University of Tehran & Tehran University of Medical Sciences (IBUTUMS) Tehran
3. Department of Immunology, Faculty of Veterinary Medicine, University of Tehran

Background: A biomimetic platform of cell membrane-coated nanoparticles for the anti-inflammatory-based treatment of atherosclerotic plaque was developed. A sulfonylurea compound as an anti-inflammasome agent was encapsulated in a nanoparticle (NP), which was coated by an immune cell membrane.

Methods: Synthesis of PLGA nanoparticles (NP) and cell membrane-coated NP (cmNP) was performed using solvent evaporation and an extrusion procedure. NPs and cmNPs were characterized by using a zeta sizer and TEM in term of size and zeta potential. The hemocompatibility of the NG was assessed by hemolysis assay. The expression level of inflammasome genes was determined by RT-qPCR assays.

Results: The size and zeta potential of the cmNP changed to 292nm and -10nm from 189.5nm and -34.1 in the core NP. In addition, the actual size of 62.5 nm with a coating layer of 5 nm was measured using TEM. The cmNP also showed a sustained release profile with a drug-loading content of about 4.7%. A decrease in gene expression levels of NLRP3 and IL-1βin LPS-primed immune cells exposed to cmNP indicated remarkable inflammation control.

Conclusion: The bio/hemocompatible, biomimetic, and anti-inflammatory cmNP can be considered as a potential platform for immunotherapy of atherosclerosis in the field of personalized medicine.

Keywords: Atherosclerosis; Biomimetic; Inflammasome; cell membrane; nanoparticle.

Anti-inflammatory efficiency of PEG-liposomal ginger in animal model of colorectal cancer

Maryam Yavari[1], Mahmoud Reza Jaafari[2], Farshad Mirzavi[2], Ghasem Mosayebi[1], Ali Ghazavi[1], Ali Ganji[1]

1. Arak University of Medical Sciences, Arak, Iran.
2. Mashhad University of Medical Sciences, Mashhad, Iran.

Background: This study aimed to develop a nano liposomal formulation containing ginger ethanolic extract with a higher therapeutic effect for cancer treatment.

Methods: The present study aimed to prepare PEGylated nanoliposomal ginger through the thin film hydration method plus extrusion. Physicochemical characteristics were evaluated. In addition, tumor size was monitored in colorectal cancer-bearing mice. Also, the anti-inflammatory effects of liposomal ginger were evaluated by gene expression assay of cytokines including TNF-α, TGF-β and IFN-γ by Real-time PCR.

Results: The nanoliposomes' particle size and polydispersity index (PDI) were 94.95 nm and 0.246 nm, respectively. High encapsulation capacity (80 %) confirmed the technique's efficiency, and the release rate of the extract was 85% at pH 6.5. In addition, this study showed that liposomal ginger at 100 mg/kg/day enhanced the expression of IFN-γ(p<0.01) compared with ginger extract in the mouse model.

Conclusion: Results indicated that the liposomal ginger enhanced the anti-inflammatory activity; therefore, the prepared liposomal ginger can be used in future clinical trials.

Keywords: colorectal cancer, ginger, liposome, Interferon-gamma

Anti-tumor efficiency of nanoliposomes encapsulated ginger in colorectal cancer-bearing mice

Maryam Yavari[1], Mahmoud Reza Jaafari[2], Farshad Mirzavi[2], Ghasem Mosayebi[1], Ali Ghazavi[1], Ali Ganji[1]

1. Arak University of Medical Sciences, Arak, Iran.
2. Mashhad University of Medical Sciences, Mashhad, Iran.

Background: This study focuses on the liposomal of ginger-targeted drug delivery in colon cancer in order to overcome the limitations of common cancer treatments.

Methods: The present study aimed to prepare PEGylated nano liposomal ginger through the thin film hydration method plus extrusion. Physicochemical characteristics were evaluated, and the toxicity of the prepared liposomes was assessed using the MTT assay. In addition, tumor size was monitored in colorectal cancer-bearing mice. Also, the anticancer effects of liposomal ginger were evaluated by gene expression assay of Bax and Bcl-2 by Real-time PCR. Also, cytotoxic T lymphocytes (CTLs) and regulatory T lymphocytes (Treg cells) were counted in the spleen and tumor tissue by flow cytometry assay.

Results: The nanoliposomes' particle size and polydispersity index (PDI) were 94.95 nm and 0.246 nm, respectively. High encapsulation capacity (80 %) confirmed the technique's efficiency, and the release rate of the extract was 85% at pH 6.5. In addition, this study showed that liposomal ginger at 100 mg/kg/day enhanced the expression of Bax (p<0.05) compared with ginger extract in the mouse model. Also, the number of tumor-infiltrating lymphocytes (TILs) and CTLs cell count in tumor tissue showed a significant increase in the LipGin group compared with the Gin group (p<0.05).

Conclusion: Results indicated that the liposomal ginger enhanced the antitumor activity; therefore, the proposed formulation might be considered a promising solution for active targeted drug delivery and can be used in future clinical trials.

Keywords: Colorectal cancer, Ginger, Liposomes, Bax protein

Biosynthesis and chemical characterization of Artemisia Dracunculus extract -capped gold nanoparticles for the treatment of acute myeloid leukemia in comparison to doxorubicin in a leukemic mouse model

Mohammad Mehdi Zangeneh[1], Navid Etemadi[2], Naser Abbasi[1], Hori Ghanei Alvar[1], Akram Zangeneh[1]

1. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.
2. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.

Background: The recent study reveals the cytotoxicity, antioxidant, and anti-acute myeloid leukemia properties of Artemisia Dracunculus extract-capped gold nanoparticles compared to doxorubicin in a leukemic mouse model.

Methods: In the biological in vivo part of this study, induction of acute myeloid leukemia was done by 7,12-Dimethylbenz[a]anthracene (DMBA) in 75 mice. Then, the animals were randomly divided into six subgroups, including control, untreated, doxorubicin, AuNPs, Artemisia Dracunculus, and AuNO3. The synthesized AuNPs were characterized using different techniques including ultraviolet–visible spectroscopy (UV–Vis.), Fourier-transform infrared spectroscopy (FT-IR) spectroscopy, and X-ray diffraction (XRD), energy Dispersive X-ray Spectrometry (EDS), field emission-scanning electron microscopy (FE-SEM), and transmission electron microscopy (TEM). FE-SEM and TEM images revealed a uniform spherical morphology and average diameters of 20–30 nm for the nanoparticles. Also, in XRD analysis, ~35 nm was measured for the crystal size of nanoparticles. The results of the biological experiments were fed into SPSS-22 software and analyzed by one-way ANOVA.

Results: In the biological in vitro part of this study, AuNPs similar to doxorubicin had low cell viability dose-dependently against Human HL-60/VCR, 32D-FLT3-ITD, and Murine C1498 cell lines without any cytotoxicity on HUVEC cell line. In this study, 2,2-diphenyl-1-picrylhydrazyl (DPPH) test revealed similar antioxidant potentials for doxorubicin and AuNPs. AuNPs similar to doxorubicin, significantly ($p \leq 0.05$) reduced the pro-inflammatory cytokines, and the total white blood cell (WBC), blast, monocyte, neutrophil, eosinophil, and basophil counts and enhanced the anti-inflammatory cytokines and the platelet, lymphocyte, and red blood cell (RBC) parameters as compared to the untreated mice.

Conclusion: Above results confirm the therapeutic effects of AuNPs on acute myeloid leukemia in leukemic mice. Further clinical trials are necessary for confirmation of these remedial properties of AuNPs in humans.

Keywords: Artemisia Dracunculus extract -capped gold nanoparticles; acute myeloid leukemia; doxorubicin

Comparative Effects of Gold and Copper Nanoparticles of Allium saralicum Aqueous Extract against fatty liver in rats

Mohammadmehdi Zangeneh[1], Samira Zand[2], Akram Zangeneh[1]

1. Biotechnology and Medicinal plants Research Center Ilam University of Medical Sciences, Ilam, Iran
2. Department of Microbiology, Faculty of veterinary Medicine, Urmia University, Urmia, Iran

Background: Accumulation of triglyceride in the liver ultimately may lead to fatty liver disease which in some individuals elicits an inflammatory response that can progress to cirrhosis and liver cancer. It is predicted that Allium saralicum (A. saralicum) leaves have significant antioxidant properties. Recently several nanoparticles such as Gold and Copper Nanoparticles platforms are currently being developed for applications in medicine due to their anti-inflammatory effects. In this regard, we designed a study to investigate the extract of A. saralicum and Gold/Copper Nanoparticles mixture and how affects the fatty liver in mice.

Methods: In this study, rats were used. A total of 24 rats were selected as the negative control, and the rest of them were treated with a high-fat diet for 4 months. Then, the animals were randomly divided into four subgroups, including negative healthy control, untreated negative control, and two groups receiving the 200 mg/kg aqueous extract of A. saralicum and a mixture of 0.5 mg/kg Gold/Copper Nanoparticles and extract of A. sacrarium. After 2 months, the rats were sacrificed and blood and liver samples of them were collected to analyze the biochemical and histopathological parameters.

Results: Our findings showed that the combination group was able to notably reduce the levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total bilirubin, urea, and creatinine and also improve the levels of HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the other treatment groups. Further, it also reduces NO, MPO, and MDA levels in plasma compared to other treatment groups.

Conclusion: The results confirm that the aqueous extract of A. saralicum leaves can be used to yield Gold/Copper Nanoparticles with a notable amount of remedial.

Keywords: fatty liver, Allium saralicum, Gold Nanoparticles, Copper Nanoparticles

Development of tumor cell lysate-based nanoparticles as a promising approach for breast cancer vaccine

Maryam Abbaspour[1], Vajihe Akbari[1], Nafiseh Esmaeil[1]

1. Isfahan University of Medical Sciences, Isfahan, Iran.

Background: Tumor cell lysate-based nanoparticles (TCL-NPs) effectively deliver tumor antigens to dendritic cells, which are the most important antigen-presenting cells that play an essential role in initiating and regulating immune responses. By exposing dendritic cells to tumor cell lysates, they can stimulate T cells to recognize tumor-associated and tumor-specific antigens and generate an immune response against cancer. However, vaccination with tumor cell lysis produces limited therapeutic efficacy with weak antitumor T cell responses. Nanoparticles or vaccines based on dendritic cells are used to solve this challenge.

Methods: TCL-NPs were generated from a 4T1 cell line with different lysate preparation methods. Dendritic cells (DCs) were isolated from mouse bone marrow and incubated with 20 ng/ml GM-CSF and 10 ng/ml IL-4 for 7 days. We evaluated the properties of the DC vaccine that was exposed to tumor lysates by five different methods in vitro. We evaluated i) DCs pulsed with freeze-thawed necrotic tumor cells, ii) DCs pulsed with HSP, iii) DCs pulsed with HOCl, iv) DCs pulsed with UV and v) DC-tumor cell fusion nanoparticles (TCL-NPs). Also, we measured the effects of tumor lysate on the maturation of DCs using the flow cytometry method, and the proliferation activity of pulsed T cells on DCs was measured.

Results: It was shown that dendritic cells treated with TCL-NPs are more, able to activate T cells and create a strong anti-tumor immune response. TCL-NPs significantly upregulated the expression level of CD 11c and CD86 in pulsed DCs. DCs exposed to TCL-NPs and DCs pulsed with HOCم were more potent than DCs with freeze-thawed necrotic tumor cells to induce protective anti-tumor responses ($p< 0.01$).

Conclusion: The use of nanoparticles in vaccines is considered a promising approach for improving vaccine efficacy and accessibility. However, more in vitro and in vivo experiments are still needed.

Keywords: Dendritic cells, Tumor cell lysate-based nanoparticles (TCL-NPs), Breast Cancer, and DC Vaccine

Enhanced anti-tumor immune responses nanoliposomes encapsulated plasmid anti-PD-11 small interference RNA in a melanoma tumor model

Mehdi Barati[1]

1. Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Programmed cell death protein-1 (PD-1), as an immune checkpoint molecule, decreases T cell activity and induces T cell exhaustion. Employing siRNA to silence the PD-1 gene, is a treatment strategy to restore immune function in cancer treatment. Although siRNA has great potential in various cancer immunotherapy, its delivery to target cells is the main limitation of its usage. This study aimed to prepare liposomal plasmid carriers of siRNA PD-1 to T CD8+ lymphocytes, in order to investigate the antitumor efficacy of siRNA PD-1.

Methods: The liposomal formulation was prepared and then characterized by size, zeta potential, and biodistribution. Following that, the uptake assay and mRNA silencing were evaluated in vitro by the detection of PD-1 in T lymphocytes at mRNA and protein levels. Tumor-infiltrated lymphocytes were harvested from B16F0 tumor-bearing mice and after expansion of T CD8+ lymphocytes, they were transfected by plasmid carrier of siRNA PD-1. The expanded T CD8+ lymphocytes were injected into B16F0 tumor-bearing mice to evaluate tumor growth, as well as tumor infiltration and survival of lymphocytes.

Results: Liposomal and also plasmid carrier of siRNA PD-1, efficiently silenced PD-1 gene expression in T lymphocytes ($p<0.0001$). T CD8+ lymphocytes transfected by plasmid carrier of siRNA PD-1 enhanced the infiltration of T helper 1 (Th 1) and cytotoxic T lymphocytes into the tumor environment ($p<0.0001$). The therapy improved the survival rate of mice and delayed their growth in the intervention groups compared to the controls ($p<0.001$).

Conclusion: Overall, these findings suggest that immunotherapy with plasmid carriers of siRNA PD-1 transfected to CD8+ T lymphocytes could enhance T cell-mediated anti-tumor immune responses and it seems that this therapy might be a promising strategy for the treatment of patients with melanoma cancer.

Keywords: Immune checkpoint, Immunotherapy, Liposome, PD-1, T lymphocyte, plasmid siRNA, melanoma

Evaluation of anti-inflammatory effects of Nilotinib-loaded Chitosan nanoparticles in mouse-bearing colorectal cancer

Rezvan Shams[1], Ali Ganji[1]

1. Department of immunology faculty of medicine University of Arak, Arak, Iran.

Background: Colorectal cancer (CRC) is the most common gastrointestinal cancer worldwide. Nilotinib has been used as a third line of treatment for gastrointestinal tumors. One of the challenges of using oral drugs including nilotinib is low bioavailability and solubility in water which could be overcome by nanoparticles. The most important advantage of chitosan compared to other nanoparticles is related to its mucoadhesive and cytotoxicity properties for cancer cells. This study focused on the synergistic anti-inflammatory effect of chitosan and Nilotinib in the treatment of CRC.

Methods: Twenty-eight female BALB/c mice (6-8weeks) were injected with C26 cells and randomly divided into four groups, positive control (pos), nilotinib (nil), chitosan (Cs, and nil/Cs. Mice were treated with nil/CS (75 mg/kg) 3 times a week for the duration of 21 days. Selected genes associated with spleen cells were evaluated by quantitative real-time PCR.

Results: There was a significant decrease between the Cs/nil and Pos groups in terms of IL6 expression. The results indicated that the levels of TNF-αwere decreased between the Cs/nil and Pos group. Statistical analysis of TGFβexpression shows a significant downward trend between pos and Cs/nil. When it comes to INFɣexpression, there was a significant increase between the Cs/nil and Pos groups.

Conclusion: Our findings illustrated that nil/cs could have a synergistic anti-inflammatory effect, which could be considered as a promising strategy for CRC treatment.

Keywords: Nilotinib, Chitosan, Colorectal cancer

Evaluation of Curcumin-Chitosan Effect on a mouse model of liver fibrosis

Pegah Hasanzadeh[1], Ghasem Mosayebi[1], Ali Ganji[2], Ali Ghazavi[3]

1. Department of Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
3. Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran
4. Traditional and Complementary Medicine Research Center (TCMRC), Department of Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran

Background: Herbal medicine is one of the most effective treatments for liver fibrosis which can lead to premature death. In recent years, curcumin has gained considerable attention for its biological activities due to its antioxidant and anti-inflammatory properties. However, the clinical usage of curcumin is limited by poor bioavailability improved by drug delivery. Among various types of nanoparticles, Chitosan has anti-inflammatory and anti-fibrotic activity. This study focused on the synergistic effects of Chitosan-loaded Curcumin on preventing the development of liver fibrosis and its anti-inflammatory effect.

Methods: Forty-two female C57 mice (18-22g) were randomly divided into six groups, positive control (pos), sham, curcumin (Cur), chitosan (Cs), Cur/Cs, and negative control (neg). Mice were intraperitoneally (IP) injected with CCl4 twice a week for 32 days to develop liver fibrosis and treated with Cur/CS (2 mg/kg) 3 times a week for the duration of one month. Selected genes associated with liver fibrosis were evaluated by quantitative real-time PCR.

Results: There was a significant increase between the Cs/Cur and pos groups (p = 0.0088), Cs/Cur and neg (p=0.0023), and Cs with Cur/Cs (p= 0.0175) in terms of INFɣexpression. Statistical analysis of TNFɑexpression shows a significant upward trend between pos and Cur/Cs (p=0.0010) and Cur with Cur/Cs (p=0.0182). The results indicate that the levels of TGFβwere decreased in neg and Cs/Cur (p=0.0005), Cur and Cs/Cur (p=0.0124), and Cs with Cs/Cur (p=0.0097) in a significant manner. Although there weren't any significant differences in IL6 levels between groups, Cs/Cur and Cs witnessed an upward trend in comparison to the pos group.

Conclusion: This study concluded that curcumin-chitosan could be used as a proper way of treatment for liver fibrosis.

Keywords: Curcumin, Chitosan, Liver fibrosis

Hepatoprotective, nephroprotective, hematoprotective, immunoprotective, and gastroduodenal protective properties effects of the silver and copper nanoparticles synthesized using the aqueous extract of Falcaria vulgaris in Wistar male rats

Akram Zangeneh[1], Mahtab Pourkamalzadeh[2], Navid Etemadi[3], Mohammad Mehdi Zangeneh[1], Naser Abbasi[1]

1. Biotechnology and Medicinal plants Research Center Ilam University of Medical Sciences, Ilam, Iran.
2. Department of Microbiology, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.
3. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.

Background: Recent studies have indicated the properties of plants and gold nanoparticles in the prevention of gastroduodenal ulcers. Falcaria vulgaris has been used in traditional medicine as a therapeutical supplement. The aim of our research was to survey the preventive property of the silver and zinc nanoparticles synthesized using the aqueous extract of Falcaria vulgaris on ibuprofen-induced gastroduodenal ulcers by investigating the biochemical, hematological, immunological, and microscopic approaches in rats.

Methods: In this study, 48 rats were used. The animals were randomly divided into six subgroups, including negative healthy control, untreated negative control, the positive control receiving omeprazole 60 mg/kg, one group receiving the aqueous extract of Falcaria vulgaris at 180 mg/kg concentrations, a group receiving the of silver nanoparticles of aqueous extract Falcaria vulgaris at 0/5 mg/kg concentrations and a group receiving the of zinc nanoparticles of aqueous extract Falcaria vulgaris at 0/5 mg/kg concentrations After 14 days, gastroduodenal ulcers were caused by ibuprofen 400 mg/kg. Four hours after oral administration of ibuprofen, the rats were sacrificed and blood, stomach, and duodenum samples of them collected for biochemical, hematological, immunological, and microscopic parameters analysis.

Results: The silver nanoparticles of aqueous extract Falcaria vulgaris could significantly ($p\leq 0.05$) reduce the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, creatinine, IL1, IL6, IL12, IL18, IFN-Y, and TNF-αand increase HDL, total protein, albumin, WBC, platelet, RBC, IL4, IL5, IL10, IL13, and IFN-αas compared to the other groups. Also, the silver nanoparticles of aqueous extract Falcaria prevented significantly ($p\leq 0.05$) gastroduodenal ulcers as compared to the other groups.

Conclusion: The obtained results indicated the hepatoprotective, nephroprotective, hepatoprotective, immunoprotective, and gastroduodenal protective properties of silver nanoparticles of aqueous extract Falcaria vulgaris.

Keywords: silver nanoparticles of aqueous extract Falcaria vulgaris; hepatoprotective; nephroprotective; hepatoprotective; immunoprotective; gastroduodenal protective

IFN-λ1- loaded PLGA nanoparticles decrease metastases in the mouse 4T1 breast tumor model

Parisa Amir Kalvanagh[1], Zahra Matloobi[2], Mohammad Taher Afshounpour[3], Sahar Ghaffari Khaligh[4], Zuhair Muhammad Hassan[1]

1. Eepartment of Immunology, Faculty of Medical Sciences, University of Tarbiat Modares
2. Department of Immunology, Faculty of Medical Sciences, University of Sabzevar
3. Department of Physical Education & Sport Sciences, Faculty of Humanities, University of Tarbiat Modares
4. Department of Pathology, Faculty of Veterinary Medicine, University of Semnan

Background: Approximately 500,000 annual deaths are attributed to breast cancer worldwide. In recent years, the delivery of anti-breast cancer drugs via nano-drug delivery systems has become a research hotspot. The present study aimed to investigate the ability of plasmid encoding IFN-λ1 (pIFN-λ1), encapsulated by PLGA nanoparticles (NPs), in tumor size and metastatic capacity reduction in the mouse 4T1 breast tumor model.

Methods: After total RNA extraction by Qiazol reagent, the cDNA of IFN-λ1 gene was synthesized. PCR was carried out with specific primers and cloned into pcDNA3.1+. Then, the pIFN-λ1 encapsulated by PLGA NPs employing the simplified double-emulsion-solvent evaporation method. Ten days after induction of the mouse 4T1 breast tumor model, treatment was done intramuscularly three times with naked pIFN-λ1 and pIFN-λ1-loaded PLGA NPs. The free-plasmid PLGA NPs were injected into the control group. The tumor size of each animal was measured by a digital caliper for up to 28 days. The 4T1 cell metastatic potential to the liver was analyzed by hematoxylin and eosin staining.

Results: The pIFN-λ1-loaded PLGA NPs were spherical with a mean diameter 380±3 nm, zeta potential −3.3±7.6 mV, encapsulation efficiency 75±5%, and loading capacity 0.83±0.06. The pIFN-λ1-loaded PLGA NPs had a significant effect on the reduction of tumor growth in animal models as compared with naked pIFN-λ1 administration. The liver histopathological images showed mice receiving pIFN-λ1-loaded PLGA NPs had very low-grade metastasis adenocarcinoma. It was mild grade in mice treated with naked pIFN-λ1 compared to severe grade in mice injected with free-plasmid PLGA NPs.

Conclusion: The pIFN-λ1-loaded PLGA NPs exhibited significant efficacy against breast tumors. It could be considered a promising candidate for future clinical trials.

Keywords: Breast cancer, IFN-λ1, PLGA, Metastases

Nanocurcumin improves Treg cell responses in moderate and severe COPD patients

Amirhossein Mardi[1], Majid Ahmadi[2], Hamed Valizadeh[3], Sanaz Abbaspour-Aghdam[4]

*1. Student Research Committee, Tabriz University of Medical Science, Tabriz, Iran * Immunology Research Center, Tabriz University of Medical Science, Tabriz, Iran*
2. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Tuberculosis and Lung Disease Research Center of Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Clinical Biochemistry and Applied Cell Sciences, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

Background: Chronic obstructive pulmonary disease (COPD) is defined as a chronic inflammatory process in the airways that results in airflow obstruction. In COPD, a decreased number of regulatory T (Treg) cells and their related factors result in a hyperinflammatory state due to stimulation of the inflammatory cells.

Methods: In the current research, we assessed the Nanocurcumin effects on the Treg cell frequencies and related factors in moderate and severe COPD patients. To evaluate the Nanocurcumin effects, 40 COPD patients (20 at the moderate stage and 20 at the severe stage) were selected and divided into Nanocurcumin and placebo.

Results: In both the Nanocurcumin and placebo groups before and after treatment, the frequency of Treg cells, the gene expression of Treg transcription factor forkhead box P3 (FoxP3), and cytokines (IL-10, IL-35, and TGF-β), as well as the serum levels of cytokines were measured. In both moderate and severe COPD patients, Nanocurcumin could significantly upregulate the Treg cells populations, the expression levels of FoxP3, IL-10, IL-35, and TGF-β as well as the serum levels of cytokines in the Nanocurcumin-treated group in comparison to the placebo group. The abovementioned factors were significantly elevated in the Nanocurcumin post-treatment compared to pre-treatment. Likewise, it has been seen no remarkable alteration in the placebo group.

Conclusion: Our results showed that in both moderate and severe patients, Nanocurcumin's effective function in a notable increase in the Treg cell number and their associated factors in the Nanocurcumin group than in the placebo group. Therefore, it would be a potential therapeutic agent in rehabilitating COPD patients.

Keywords: COPD, T regulatory, anti-inflammatory effects, nano curcumin

Nanocurcumin modulate Th17 cell responses in moderate and severe COPD patients

Amirhossein Mardi[1], Majid Ahmadi[2], Sanaz Abbaspour-Aghdam[3], Hamed valizadeh[4]

1. Student Research Committee, Tabriz University of Medical Science, Tabriz, Iran
2. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Clinical Biochemistry and Applied Cell Sciences, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
4. Tuberculosis and Lung Disease Research Center of Tabriz University of Medical Sciences, Tabriz, Iran

Background: COPD is defined as a chronic inflammatory process in the airways that results in airflow obstruction. It is mostly linked to cigarette smoke exposure. Th17 cells have a role in the pathogenesis of COPD by secreting pro-inflammatory cytokines, which cause hyperinflammation and progression of the disease. The aim of the present study was to examine the potential therapeutic effects of nano curcumin on the Th17 cell frequency and its responses in moderate and severe COPD patients.

Methods: In this study, 20 patients with severe COPD who were hospitalized in the intensive care unit and 20 patients with moderate COPD were included. Th17 cell frequency, gene expression of Th17 cell-related factors (RAR-related orphan receptor t (RORγt), IL-17, IL-21, IL-23, and granulocyte-macrophage colony-stimulating factor), and serum levels of Th17-related cytokines were assessed before and after treatment in both placebo and nano curcumin-treated groups using flow cytometry, real-time PCR, and ELISA, respectively.

Results: According to our findings, in moderate and severe COPD patients treated with nano curcumin, there was a substantial reduction in the frequency of Th17 cells, mRNA expression, and cytokines secretion level of Th17 cell-related factors compared to the placebo group. Furthermore, after treatment, the above-mentioned metrics were considerably lower in the nano curcumin-treated group compared to before treatment. Nanocurcumin has been shown to reduce the number of Th17 cells and their respective inflammatory cytokines in moderate and severe COPD subjects.

Conclusion: As a result, it might be used as an immune-modulatory agent to alleviate the patient's inflammatory state.

Keywords: COPD, Th17, nanocurcumin, inflammatory cytokines

Nanotechnology: a potential treatment for immune wounds

Soheil Sadr[1], Hamid Reza Chaboki[2], Farideh Akbarian[3], Hossein kazemi mehrjerdi[4]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Immune factors can also cause skin wounds, which is why people with immune skin diseases frequently have ulcers. For example, allergic vasculitis is accompanied by NF-B pathway activation and increases in IL-6 and TNF, whereas dermatomyositis exhibits a proinflammatory phenotype with increased amounts of IL-10 and IL-6 after TLR7 activation. Because immune diseases are the main cause of wound formation in these circumstances, immunotherapy is essential for wound healing. In this systematic review, the anti-inflammatory capabilities of nanomaterials were discussed.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on the potential treatment of nanomaterials for immune wounds from January 2000 to January 2023. Fifteen affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: AgNPs and nanofibers can decrease IL-6, IL-1, and TNF- expression, while metal nanomaterials, including TiO2 NPs and ZnO NPs, can inhibit the NFB and TLR pathways by activating the arginase 1 and transcription factors PPAR. Nanomaterials support the phenotypic transformation of macrophages and regulate the expression of inflammatory factors in the wound during the immunomodulatory stage. Treg cells aid in the maintenance of peripheral immune tolerance in the body by secreting anti-inflammatory factors or exosome vesicles that promote T cell apoptosis and suppress T cell proliferation.

Conclusion: As a consequence, it is possible to hypothesize that nanomaterials could play a role in regulating immunological skin wound inflammation through the aforementioned pathways, but more research is required.

Keywords: Immune wounds, Immunomodulatory, Nanotechnology, Systematic review

Novel green synthesis of Matricaria chamomilla extract conjugated gold nanoparticles with excellent anti-acute myeloid leukemia effect in comparison to daunorubicin in a leukemic rodent model

Navid Etemadi[1], Mohammad Mehdi Zangeneh[2], Akram Zangeneh[3], Naser Abbasi[4]

1. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.
2. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.

Background: In this regard, pharmacologists have tried to synthesize many supplements and drugs from nanoparticles. The present study confirms the ability of the aqueous extract of Matricaria chamomilla grown under in vitro conditions for the biosynthesis of gold nanoparticles (AuNPs).

Methods: In vivo design, induction of acute myeloid leukemia was done by 7, 12-Dimethylbenz[a]anthracene (DMBA) in 75 mice. Then, the animals were randomly divided into six subgroups, including HAuCl4, M. chamomilla, AuNPs, daunorubicin, untreated, and control. These nanoparticles were characterized by fourier-transform infrared spectroscopy (FT-IR) spectroscopy, ultraviolet–visible spectroscopy (UV–Vis.), X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), and transmission electron microscopy (TEM) analysis.

Results: By quantitative real-time polymerase chain reaction, sphingosine-1-phosphate receptor-1 and sphingosine-1-phosphate receptor-5 mRNA expression in lymphocytes were significantly ($p \leq 0.05$) raised by treating the leukemic mice with the AuNPs and daunorubicin. AuNPs similar to daunorubicin, significantly ($p \leq 0.05$) reduced the pro-inflammatory cytokines (IL1, IL6, IL12, IL18, IFNY, and TNFα), and the total white blood cell (WBC), blast, monocyte, neutrophil, eosinophil, and basophil counts and enhanced the anti-inflammatory cytokines (IL4, IL5, IL10, and IFNα) and the platelet, lymphocyte, and red blood cell parameters as compared to the untreated mice.

Conclusion: The results of chemical characterization confirm that M. chamomilla can be used to produce gold nanoparticles with a remarkable amount of anti-acute myeloid leukemia effect.

Keywords: daunorubicin, acute myeloid leukemia, gold nanoparticles, Matricaria chamomilla

Preparation of nanoliposomes containing rosemary alcoholic extract with polyethylene glycol coating

Rouhollah Hemmati Bushehri[1], Mahmoud Reza Jaafari[2], Ghasem Mosayebi[3], Ali Ghazavi[4], Ali Ganji[3]

1. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
2. Nanotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran, Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran.
4. Department of Immunology & Microbiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran, Traditional and Complementary Medicine Research Center (TCMRC), Arak University of Medical Sciences, Arak, Iran.

Background: Throughout history, plants have been the basis and origin of medical treatments, and today in modern medicine, many compounds derived from plants are used as the main basis of drugs. Herbal medicines have the advantages of accessibility, strengthening of the immune system, fewer side effects, and reduction of drug resistance. Rosemary is a member of the Lamiaceae family with antitumor properties; however, low water solubility and impaired bioavailability are limiting issues in using rosemary extract. Liposomes are synthetic vesicles with permeability, bioavailability, and lack of immunogenicity and toxicity, allowing the delivery of various drugs. The aim of this study was to the preparation of liposomes and encapsulation of rosemary alcoholic extract with 7 different ratios (F1-F7) of rosemary extract, cholesterol, HSPC (High Saturated Phosphatidyl Choline), and mPED200 (Polyethylene glycol) by Thin Film Hydration method.

Methods: After preparation of lipid film, hydration with sucrose-histidine buffer, and extrusion of liposomes, size, and zeta potential were measured. After that, the encapsulation percentage and release rate of the extract from the liposome was determined.

Results: F1 was clear, filtration and extrusion were done well and did not precipitate. F2 was not transparent after sonication and became cloudy. F3 was unstable due to cholesterol depletion being turbid. In F4 and F5, excessive precipitation was observed. F6 and F7 did not pass the 100 nm filter and the size was not good.

Conclusion: According to the results obtained from different formulations, F1 is the best formulation for the preparation of liposome-containing rosemary alcoholic extract due to its clarity, good filtration, and no precipitate formation.

Keywords: Liposomes, Rosemary, Herbal

Preparation, formulation, and chemical characterization of gold nanoparticles using Allium saralicum aqueous extract for the treatment of acute myeloid leukemia in vitro and in vivo conditions

Akram Zangeneh[1], Mohammad Mehdi Zangeneh[1], Naser Abbasi[1], Navid Etemadi[2], Huri Ghanei Alvar[1]

1. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.
2. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.

Background: Synthesis of nanoparticles from various biological systems has been reported, but among all, biosynthesis of nanoparticles from plants is considered as the most suitable method. The current study confirms the potential of aqueous extract of Allium saralicum grown under in vitro condition for the green synthesis of gold nanoparticles (AuNPs). Also, we revealed the cytotoxicity, antioxidant, and anti-acute myeloid leukemia effects of AuNPs compared to mitoxantrone in a leukemic mouse model.

Methods: In vivo experiment, induction of acute myeloid leukemia was done by DMBA in 75 mice. The obtained results were fed into SPSS-22 software and analyzed by one-way ANOVA. These nanoparticles were characterized by FT-IR, UV, XRD, FE-SEM, TEM, and AFM.

Results: AuNPs similar to mitoxantrone, significantly ($p\leq$ 0.01) enhanced the platelet, lymphocyte, and RBC parameters and the anti-inflammatory cytokines (IL4, IL5, IL10, IL13, and IFNα) and reduced the total WBC, blast, monocyte, neutrophil, eosinophil, and basophil counts and the pro-inflammatory cytokines (IL1, IL6, IL12, IL18, IFNY, and TNFα) as compared to the untreated mice. By quantitative real-time PCR, S1PR1 and S1PR5 mRNA expression in lymphocytes were significantly ($p\leq$ 0.01) increased by treating the leukemic mice with the AuNPs and mitoxantrone. In vitro experiment, AuNPs similar to mitoxantrone had low cell viability dose-dependently against murine C1498, human HL-60/vcr, and 32D-FLT3-ITD cell lines without any cytotoxicity on HUVEC cell line.

Conclusion: Above results approve the excellent anti-acute myeloid leukemia, cytotoxicity, and antioxidant properties of AuNPs compared to mitoxantrone.

Keywords: acute myeloid leukemia, mitoxantrone, gold nanoparticles, Allium saralicum

Preparation, formulation, and chemical characterization of zinc nanoparticles using Falcaria vulgaris aqueous extract for the treatment of acute myeloid leukemia in vitro and in vivo conditions

Mohammad Mehdi Zangeneh[1], Huri Ghanei Alvar[1], Akram Zangeneh[1], Navid Etemadi[2], Naser Abbasi[3]

1. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.
2. Veterinary graduate, Faculty of veterinary Medicine, Urmia University, Urmia, Iran.
3. Biotechnology and Medicinal plants Research Center Ilam University of medical sciences, Ilam, Iran.

Background: we revealed the cytotoxicity, antioxidant, and anti-acute myeloid leukemia effects of ZnNPs compared to mitoxantrone in a leukemic mouse model.

Methods: In vivo experiment, induction of acute myeloid leukemia was done by DMBA in 75 mice. The obtained results were fed into SPSS-22 software and analyzed by one-way ANOVA. The synthesized ZnNPs were characterized using several techniques including UV–Vis., FT-IR, TEM, FE-SEM, and EDS.

Results: By quantitative real-time PCR, S1PR1 and S1PR5 mRNA expression in lymphocytes were significantly ($p \leq 0.01$) increased by treating the leukemic mice with the Zn NPs and mitoxantrone. Also, ZnNPs similar to mitoxantrone, significantly ($p \leq 0.01$) enhanced the platelet, lymphocyte, and RBC parameters and the anti-inflammatory cytokines (IL4, IL5, IL10 and IFNα) and reduced the total WBC, blast, monocyte, neutrophil, eosinophil, and basophil counts and the pro-inflammatory cytokines (IL12, IL18, IFNY, and TNFα) as compared to the untreated mice. In vitro experiment, Zn NPs similar to mitoxantrone had low cell viability dose-dependently against murine C1498, human HL-60/vcr, and 32D-FLT3-ITD cell lines without any cytotoxicity on HUVEC cell line. The DPPH assay showed similar antioxidant potentials for ZnNPs and mitoxantrone.

Conclusion: Above results approve the excellent anti-acute myeloid leukemia, cytotoxicity, and antioxidant properties of ZnNPs compared to mitoxantrone.

Keywords: zinc nanoparticles using Falcaria vulgaris; acute myeloid leukemia; mitoxantrone

The copper and zinc nanoparticles synthesized using the aqueous extract of Artemisia (Mugworts) effectively prevent induced ulcerative colitis

Neda Ahmadiafshar[1], Somayeh Ahmadiafshar[2], Huri Ghaneialvar[3], Mohammad Mehdi zangeneh[2], Akram zangeneh[2]

1. Tehran University of Medical Sciences, Tehran, Iran.
2. Urmia University of Medical Sciences, Urmia, Iran.
3. Ilam University of Medical Sciences, Urmia, Iran.

Background: Nanotechnology has improved medical science in the diagnosis and monitoring of disease and treatment. The aim of this study was to investigate the antioxidant activities and protective effect of copper and zinc nanoparticles against ulcerative colitis in rats.

Methods: In this study, 40 adult male rats were divided into 5 groups, randomly: Healthy control group, Patient control group, Patient group receiving Prednisolone (60 mg/kg), Patient group receiving Artemisia (Mugworts) aqueous extract (200mg/kg), Patient group receiving copper and zinc nanoparticles of Artemisia (Mugworts) aqueous extract (0/5 mg/kg). To induce ulcerative colitis, acetic acid was injected intra rectally. After confirming the induction of the disease, the treatment period was started in rats. After ten days, the plasma and serum of rats were sent for hematological and biochemical tests, and intestines of rats for histopathology.

Results: The results showed that the level of AST, ALT, ALP, GGT, cholesterol, LDL, triglyceride, Creatinine, HDL, total protein, WBC, hemoglobin, RBC, PCV, MCV, MCH and MPO, MDA, NO and also necrosis, erythrocyte congestion and accumulation of inflammatory cells in the intestinal tract were significantly reduced in the group receiving nanoparticles compared to other groups. On the other hand, macroscopic examination of tissues showed a significant decrease in the level of all three types of small, large and medium wounds in this group compared to other groups.

Conclusion: This study showed that the use of copper and zinc nanoparticles containing Artemisia (Mugworts) extracts has significant antioxidant and protective effects compared to the use of this plant alone or the use of prednisolone in the treatment of ulcerative colitis in rats.

Keywords: Ulcerative Colitis, Copper and Zinc Nanoparticles, Artemisia, Rat

The copper and zinc nanoparticles synthesized using the aqueous extract of Artemisia dracunculus effectively prevent induced gastroduodenal ulcer.

Akram Zangeneh[1], Mohammad Mehdi Zangeneh[2], Somayeh Ahmadiafshar[2], Naser Abbasi[2], Neda Ahmadiafshar[3], Mohammad Abbaszade[2]

1. *Urmia University of Medical Sciences, Urmia, Iran.*
2. *Ilam University of Medical Sciences, Urmia, Iran*
3. *Tehran University of Medical Sciences, Tehran, Iran.*

Background: Many people in the world suffer from gastroduodenal ulcers, therefore, studying the therapeutic strategies of these ulcers are research priority in any country. The aim of the new study was to survey the preventive property of copper and zinc nanoparticles of Artemisia dracunculus on ethanol-induced gastroduodenal ulcers in rats.

Methods: In this study, 35 adult male rats were divided into ۵ groups, randomly: negative healthy control receiving distilled water, untreated negative control receiving distilled water, positive control receiving omeprazole ۴۰mg/kg, one group receiving the aqueous extract of Artemisia dracunculus at 200 mg/kg concentrations, and a group receiving the of copper and zinc nanoparticles of aqueous extract Artemisia dracunculus at 5 mg/kg concentrations. After ۱۴ days, gastroduodenal ulcers were caused by ethanol. Four hours after oral administration of ethanol, the rats were dissected and blood, stomach, and duodenum samples of them collected for hematological, biochemical, Immunology, and gross parameters analysis.

Results: The copper and zinc nanoparticles of aqueous extract Artemisia dracunculus could significantly decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the Other groups. Also, the copper and zinc nanoparticles of aqueous extract prevented significant small, medium, and large gastroduodenal ulcers as compared to the other groups. It also further reduces NO, MPO, and MDA compared to other groups.

Conclusion: It seems that the copper and zinc nanoparticles of aqueous extract Artemisia dracunculus can prevent gastroduodenal ulcers in rats without any side effects.

Keywords: copper and zinc nanoparticles, Artemisia dracunculus, gastroduodenal ulcer, Rat

The silver and zinc nanoparticles synthesized using the aqueous extract of Matricaria chamomilla effectively prevent induced gastroduodenal ulcers.

Naser Abbasi[1], Somayeh Ahmadiafshar[2], Mohammad Abbaszadeh[1], Mohammad Mehdi zangeneh[1], Samira Zand[2], Akram zangeneh[2], Neda Ahmadiafshar[3]

1. Ilam University of Medical Sciences, Urmia, Iran
2. Urmia University of Medical Sciences, Urmia, Iran.
3. Tehran University of Medical Sciences, Tehran, Iran.

Background: Gastroduodenal ulcer is a very common illness in the world. Therefore, studying the therapeutic strategies of these ulcers are of great importance. The aim of the new study was to survey the preventive property of silver and zinc nanoparticles of Matricaria chamomilla on ethanol-induced gastroduodenal ulcers in rats.

Methods: In this study, 35 adult male rats were divided into 5 groups, randomly: negative healthy control receiving distilled water, untreated negative control receiving distilled water, positive control receiving omeprazole 60 mg/kg, one group receiving the aqueous extract of Matricaria chamomilla at 200 mg/kg concentrations, and a group receiving the of silver and zinc nanoparticles of aqueous extract Matricaria chamomilla at 5mg/kg concentrations. After 14 days, gastroduodenal ulcers were caused by ethanol. Four hours after oral administration of ethanol, the rats were dissected and blood, stomach, and duodenum samples of them collected for hematological, biochemical, Immunology, and gross parameters analysis.**Results**: The silver and zinc nanoparticles of aqueous extract Matricaria chamomilla-could significantly decrease the raised levels of ALP, AST, ALT, GGT, cholesterol, LDL, triglyceride, total and conjugated bilirubin, urea, and creatinine and enhance HDL, total protein, albumin, WBC, platelet, RBC, Hb, PCV, MCV, MCH, and MCHC as compared to the Other groups. Also, the silver and zinc nanoparticles of aqueous extract prevented significantly small, medium and large gastroduodenal ulcers as compared to the other groups. It also further reduces NO, MPO, and MDA compared to other groups.

Conclusion: It seems that the silver and zinc nanoparticles of the aqueous extract Matricaria chamomilla prevent gastroduodenal ulcers in rats without any side effects.

Keywords: silver and zinc nanoparticles, Matricaria chamomilla, gastroduodenal ulcer, Rat

Nutrition and Immunology

Study of zinc supplement on antioxidative genes in multiple myeloma patients undergo autologous hematopoietic stem cell transplantation compared with placebo

Kasra Jahankhani[1], Niloofar Taghipour[2], Hossein Behboudi[3], Maryam Nikoonezhad[4], Mahshid Mehdizadeh[5], Abbas Hajifathali[5], Nariman Mosaffa[6]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Faculty of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran
4. Department of Immunology, School of Medicine, Tarbiat Modarres University, Tehran, Iran
5. Hematopoietic stem cell research center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
6. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Disorders caused by oxidative stress in many cancers are important factors in inflammation and treatment failure. In hematological cancers, especially multiple myeloma (MM), there is a high level of oxidative stress and even after bone marrow transplantation, it shows irreversible complications. Administration of preventive supplements plays a role in controlling and inhibiting the action of oxidative radicals. The aim of this study was to evaluate the effect of oral supplements on improving the treatment process of patients with a history of MM who have undergone autologous bone marrow transplantation in terms of changes in antioxidative genes such as NRF2 and SOD1.

Methods: Autologous bone marrow transplant candidates who were suffering from MM, were selected for this study. Patients were randomly divided into two groups receiving zinc and placebo and received 30 mg zinc tablets daily from day1 after transplantation until 1 month later. Blood samples were taken from patients in 3 periods including admission day, +15 and +30 days after transplantation. Peripheral blood mononuclear cells isolated for RNA extraction and gene expression. The gene expression level of oxidative and anti-oxidative genes including SOD1 and NRF2, were measured by Real time PCR.

Results: The results showed that the expression levels of SOD1 and NRF2 genes were significantly different in both groups receiving zinc and placebo. These 2 genes had a significant increase on days 15 and 30 compared to the control ($p<0.05$) and showed a greater increase in the expression of these 2 genes on day 30 compared to day 15 ($p<0.05$).

Conclusion: These findings showed that zinc upregulates the activity of Nrf2 and SOD1. Therefore, the regulation of Nrf2 and SOD1 by "zinc" may act as an anti-oxidative agent in the body. Therefore, zinc supplementation can be useful in modulating the level of oxidative stress and inflammation in MM patients.

Keywords: Multiple myeloma, zinc, Oxidative Stress, Bone Marrow Transplantation

Association between IgE levels and continuous or temporary course of food allergy treatment in children with atopic dermatitis: a systematic review

Zohreh Farrokhian[1], Mahdi Karimi[1], Fateme Pakbaz[1]

1. Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: Food allergies in children disappear with time. However, little is known about the predictors for the persistence of food allergy in children with atopic dermatitis. The purpose of this study is to determine the association between IgE levels and the continuous or temporary course of food allergy treatment in children with atopic dermatitis.

Methods: We systematically searched the PubMed website from January 1, 1996, to April 30, 2022, for studies investigating children with food allergy and atopic dermatitis.

Results: We identified one hundred sixty-eight studies on children with atopic dermatitis and food allergy. Fourteen relative publications were eligible for systematic review. Finally, after applying the inclusion and exclusion criteria, five clinical trials and randomized clinical trials were found and the following results were obtained from children with food allergy and atopic dermatitis: In all studies, we assigned IgE levels for the six specific foods and check their ability to predict decisive food allergy. IgE levels were predictable at the beginning of treatment (p all <0.05). In one study, a comparison of the two groups showed that IgE as well as total IgE in serum were significantly higher in the latter group. However, looking at the time course, IgE did not decrease significantly during the elimination diet. The atopy patch test and T cell levels seem to be valuable additional tools in the diagnostic.

Conclusion: Our results show that, although IgE in serum is very useful at the time of the first diagnosis, it cannot predict that IgE levels will be effective in diagnosing the treatment process in the long term. Therefore, it is suggested to conduct an interventional study with a larger population and a combination of different tests to predict the treatment process of children with atopic dermatitis food allergy.

Keywords: Atopic dermatitis, Food allergy, Children

Changes in MCP-1, HGF, and IGF-1 expression in endometrial stromal cells, PBMCs, and PFMCs of endometriotic women following 1,25(OH)2D3 treatment

Sahel Heidari[1] , Ali-Akbar Delbandi[1]

Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: 1, 25$(OH)_2$-D3 has anti-inflammatory and growth inhibitory effects.

Methods|: Our study explored the effect of 1, 25$(OH)_2$-D3 treatment on the expression of monocyte chemotactic protein-1 (MCP-1), hepatocyte growth factor (HGF), and insulin-like growth factor-1 (IGF-1) by peripheral blood mononuclear cells (PBMCs), peritoneal fluid mononuclear cells (PFMCs), endometrial stromal cells (ESCs), and its effect on the proliferation of PBMCs and PFMCs of patients with endometriosis compared with controls. PBMCs, PFMCs, and ESCs were obtained from 10 endometriosis patients and 10 non-endometriotic individuals. After treating cells with 0.1 µM of 1,25(OH)2D3 for 6, 24, and 48 h, the gene and protein expression of mentioned factors were evaluated by real-time PCR and ELISA methods, respectively.

Results: 1, 25$(OH)_2$-D3 treatment significantly reduced the protein expression of MCP-1, HGF, and IGF-1 in PBMCs and PFMCs of endometriotic patients at 48 h ($p < 0.05$-<0.01). Also, this treatment significantly reduced MCP-1, HGF, and IGF-1 gene and/or protein expression in EESCs and EuESCs at 24 and 48 h ($p < 0.05$-<0.01). 1, 25$(OH)_2$-D3 treatment also reduced the proliferation of PBMCs and PFMCs of endometriotic patients compared with controls ($p < 0.01$).

Conclusion: 1, 25$(OH)_2$-D3 can be considered as a potentially effective agent in the prevention and treatment of endometriosis along with other therapies.

Keywords: 1, 25(OH)2D3; HGF; IGF-1; MCP-1; endometrial stromal cells; endometriosis; mononuclear cells.

Effect of curcumin on inflammatory biomarkers in patients with premenstrual syndrome and dysmenorrhea: A randomized clinical trial

Afsane Bahrami[1, 2], Mansoore Saharkhiz[1, 2]

1. *Immunology Department, Birjand University of Medical Sciences, Birjand, Iran.*
2. *Cellular and Molecular Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

Background: Inflammation may have a role in the etiology of premenstrual syndrome (PMS) and primary dysmenorrhea. There is increasing evidence for the anti-inflammatory effects of curcumin, a polyphenolic natural product. This study assessed the effects of curcumin on inflammatory biomarkers, cytokines and iron profile in young women with PMS and dysmenorrhea.

Methods: A sample of 76 patients was included in this triple-blind, placebo-controlled clinical trial. Participants randomly allocated to curcumin (n=38) and control groups (n=38). Each participant received one capsule (500 mg of curcuminoid+ piperine, or placebo) daily, from 7 days before until 3 days after menstruation for three consecutive menstrual cycles. Serum iron, ferritin, total iron-binding capacity (TIBC) and high-sensitivity C-reactive protein (hsCRP), as well as white blood cell, lymphocyte, neutrophil, platelet counts, mean platelet volume (MPV) and red blood cell distribution width (RDW), were quantified. Neutrophil: lymphocyte ratio (NLR), platelet: lymphocyte ratio (PLR), and RDW: platelet ratio (RPR) were also calculated. Serum IL-10 and IL-12 levels were quantifid by an ELISA kit.

Results: Curcumin significantly decreased the median serum levels of hsCRP [from 0.30 mg/l (0.0-1.10) to 0.20 mg/l (0.0-1.3); P=0.041] compared with placebo, but did not show any difference for neutrophil, RDW, MPV, NLR, PLR and RPR values (p>0.05). None of markers of iron metabolism statistically changed after the intervention in the curcumin group (p>0.05). Serum concentrations of IL-10 and IL-12 before and after the trial period did not differ signifiantly between the two groups (p> 0.05)

Conclusions: Curcumin supplementation might be have positive effect on hsCRP with no any changes on iron homeostasis in healthy women with PMS and dysmenorrhea.

Keywords: Curcumin, Inflammation, premenstrual syndrome, dysmenorrhea

Effect of Curcuminoid on systemic immunity and radical scavenging activity in women with premenstrual syndrome and dysmenorrhea: A post-hoc analysis of a randomized clinical study

Saeid Rezaei[1], Malaksima Ayadilord[2], Afsane Bahrami[3]

1. The Five Senses Institute, Rassoul Akram Hospital, Iran University of Medical Sciences, Sattarkhan Niayesh St., Tehran, Iran.
2. Department of Medical Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Cellular and Molecular Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Oxidative stress and inflammation are in the pathoetiology of menstrual associated complications. Curcuminoids, are polyphenolic natural compounds that have potentially important functional activities, including antioxidant properties. This randomized, triple-blind, placebo-controlled trial was performed to investigate the effects of a curcuminoids on inflammation, oxidative stress and antioxidant capacity in female with premenstrual syndrome (PMS) and dysmenorrhea.

Methods: Eighty young girls with both PMS and dysmenorrhea were randomly receive either curcuminoids (500mg + 5mg piperine) or placebo daily, for a period from 7 days pre- till 3 days post- initiation of menstrual bleeding for 3 successive menstrual cycles. Anthropometrical, and biochemical characteristics, as well as dietary intake of participants were assessed at baseline and post intervention. The total antioxidant capacity and free radical scavenging activity of serum and urine were quantified via ferric reducing/antioxidant power (FRAP) and α, α-diphenyl-β-picrylhydrazyl (DPPH) methods, respectively. Malondialdehyde (MDA), as a marker of oxidative stress, was also measured. NOx (nitrite + nitrate) was measured by using the Griess assay.

Results: At baseline, no significant differences were detected between the placebo and curcumin groups, concerning to the age, dietary intake and biochemical/anthropometric indices (p>0.05). Curcumin significantly promoted the free radical scavenging activity of serum versus placebo (p=0.031). Although, no significant changes were found in serum and urinary levels of FRAP, DPPH and MDA between the groups (p>0.5). Also, a significant decrement was found in serum concentrations of NOx (93.3±37.4 to 85.9±28.9; p=0.048) after curcuminoid supplementation, but not for the placebo group, NOx levels remained unaltered by the end of trial (72.4±42.5 to 68.4±32.9; p=0.32).

Conclusion: Curcumin improves radical scavenging activity and antioxidant potential and it also reduces NOx as an inflammatory marker in women with PMS and dysmenorrhea. Investigations with higher doses and duration of curcumin are required to verify our findings.

Keywords: Malondialdehyde; ferric reducing/antioxidant power; α-diphenyl-β-picrylhydrazyl; Menstruation; Pain

Effect of elimination diet on IgE levels and allergic reactions in eosinophilic esophagitis patients: a systematic review

Mahdi Karimi[1], Fateme Pakbaz[1], Zohreh Farrokhian[1]

1. Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: Eosinophilic esophagitis (EoE) is an esophageal disorder caused primarily by food antigens. The "Elimination Diet" can help by eliminating food antigens. The aim of this study is to investigate the effect of an elimination diet on IgE levels and allergic reactions in patients with eosinophilic esophagitis.

Methods: Scientific reports were searched on the PubMed website from October 1, 2005, to September 30, 2022, for studies of an elimination diet in eosinophilic esophagitis patients.

Results: One hundred and seventy-nine studies on eosinophilic esophagitis patients and elimination diet were identified. Twenty-five relative publications were eligible for systematic review. Finally, after applying the inclusion and exclusion criteria, 6 clinical trials and randomized clinical trials were found and the following results were obtained from eosinophilic esophagitis patients: Generally, the higher levels of IgE for a given food, the more likely it is that the patient has a clinical allergy to that food. A FFGED (Four Food Group Elimination Diet) achieved clinicopathological improvement in 54% of patients with EoE. A SFGED (Six Food Group Elimination Diet) was effective in approximately one-third of FFGED non-responders, resulting in a combined effectiveness of 72% of both strategies. All patients who continued to avoid offending foods maintained histopathological and clinical remission of EoE for up to 3 years. In all studies common symptoms of EoE included dysphagia (96%), food retention (74%) and heartburn (94%) decreased by 94% (P all < .0001).

Conclusion: We conclude that in EoE, any treatment involving an elimination diet is effective and the levels of IgE associated with the elimination diet are reduced. Thus, the risk of immediate complications is decreased, and the patient's quality of life is improved. However, it is suggested that larger interventional studies should be conducted considering other diagnostic tests and report more accurate results.

Keywords: elimination diet, eosinophilic esophagitis, IgE

Effect of pomegranate extract on human blood lymphocytes

Arefeh Majidi Khameneh[1], Nafise Paysokhan[2], Zahra Matluobi[2]

1. Student Research Committee, Sabzevar University of Medical Sciences, Sabzevar, Iran 2- Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran
2. Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran

Background: Today, research on the properties of herbal medicine has been an interesting topic in medical science. One of these topics is related to their effect on the immune system. One of these plants is the pomegranate. According to traditional medicine, pomegranate extract can stimulate the immune system and improved it. In this research, this theory has been tried to be investigated.

Methods: First lymphocyte cells were separated from fresh blood using Ficoll and were cultured in 24 wells plate with RPMI media, supplemented with 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. Then, pomegranate extract was added to wells with concentrations of 250µl per 1ml (1:4) and100µl per 1ml (1:10). Cells were maintained in a humidified incubator at 37°C with 5% CO2. After 24 hours the MTT assay test was performed on it.

Results: The results of the MTT test showed that the treatment with sterilized pomegranate extract (using filter 220 µm) caused the growth of lymphocytes. Interestingly, by increasing the amount of pomegranate extract, the proliferation rate of lymphocytes also had increased. At a concentration of 250 µl per 1 ml (1:4), 167% increase in growth, and at a concentration of 100µl per 1 ml (1:10), 70% growth in lymphocytes was observed.

Conclusion: The results obtained from this study indicated that despite being sterile, pomegranate extract stimulated the immune system and was caused the division of lymphocyte cells. Although more research is needed.Keywords: pomegranate extract, herbal medicine, lymphocytes.

Keywords: pomegranate extract, herbal medicine, lymphocytes

Evaluation of the relationship between consumed food and the prevalence of asthma and allergy symptoms in children and adolescents in Shiraz City

Mahsa Shekari[1], Zeinab Sadat Moosavifard[2], Arash Fathi[1]

1. Bachelor of Nursing student, Young and Elite Researchers Club, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran
2. Faculty, Department of Nursing, Bandar Abbas Branch, Islamic Azad University, Bandar Abbas, Iran

Background: Asthma is one of the respiratory diseases in which a person's airways become narrow and it becomes difficult for a person to breathe due to coughing. This disease is among the diseases for which no specific treatment has been found, but with certain controls, including diet control, the symptoms of the disease can be minimized. The prevalence of asthma and other allergic diseases has been increasing over the past three decades. Genetic factors do not justify the increase in allergies during this period, and several studies have suggested the possibility of a relationship between environmental factors, including diets, and the increase in the prevalence of these diseases. This research was carried out with the aim of determining the relationship between the consumption of different types of food and the amount of each consumption with the prevalence of the symptoms of these diseases in the year 1400 in Shiraz city.

Methods: This is a descriptive-analytical research that was conducted using a cross-sectional descriptive method on 1500 children aged 7-9 years from the first to third year of primary schools and 2000 adolescents from 15-17 years old in secondary schools in Shiraz. The samples were randomly entered into the study and written consent was obtained from the students' families. The data was collected by Isak standard questionnaire and the questionnaires were analyzed by SPSS.

Results: In the age group of 7-9 years, the prevalence of symptoms of shortness of breath and wheezing, allergic rhinitis, eczema, and skin itching were 8.4%, 23.3%, and 7.7%, respectively, and in the age group of 15-17 years, 26.3%, 39.5%, and 7.7%, respectively. It is 19.5%. In children aged 7-9, there is a significant relationship between the high consumption of peanuts, eggs, nuts, animal butter, chocolate, sweets, puffs and chips with an increase in the frequency of allergic rhinitis. In adolescents aged 15-17, there is a significant relationship between the frequency of wheezing symptoms and high consumption of foods such as shrimp and fish, peanuts, nuts, chocolate, puffs, chips, eggs, and beverages.

Conclusion: The consumption of some foods, including convenience foods such as puffs and chips, in children and adolescents is associated with an increase in the symptoms of asthma, allergic rhinitis and eczema. In order to prevent the development of asthma symptoms and also to move on the path of recovery, it is better to prevent the occurrence of asthma symptoms by doing activities in addition to not consuming foods harmful to asthma. For example, continuous exercise based on the exercise program can be effective in treating asthma and preventing the symptoms of this disease.

Keywords: Food, asthma, allergy symptoms, children, adolescents

Food Allergy; it is not to be sneezed at!

Lotfollah BehrooJaaniaar[1], Negin Noori[2], Ali Misaghi[3]

1. Department of Food Hygiene and Quality Control, Faculty of Veterinary, University of Tehran, Iran.

Background: Neatly-talking, while some substances present in food may be nutritional and/or gratifying, they may not-necessarily be safe for any individual. In the other words, under a given-condition, an exposure to a food/food-additive or even, a food-component which is formed in-situ at the time of preparing/cooking, is followed clinically, by an abnormal immune-response referred to as –FOOD ALLERGY- that according to its acuity, has alternatively threatened beings'-lives worldwide since long. A food allergy, by definition, is an acquired-condition characterized by an exaggerated/reproducible adverse immune-reaction against a certain food-protein(s) with acute-onset of symptoms-effusion.

Methods: In the field of clinical-medicine and in line with any expedient-enterprises are lending themselves to pave the way for health-care policies/purposes -in terms of intervening, preventing and even, treating of several human ailments-, the major emphasis is being placed on the utilization of medicinal-plants. Numerous medicinal-plants, thanks to have a variety of natural/nutraceutical components with antioxidant/free-radical-scavenging/anti-inflammatory/anti-allergic properties, still serve as leads for the development/progression of novel pharmaceutical agents/preparations.

Results: Irrespective of just a few interlocutory/polemical findings of a myriad of researches seeking incessantly, after the most effective treatment for food allergy, there is, as yet, almost no conclusive curative-care for it. Adversely, the prevalence rate of food allergy is drastically on the upgrade, day in and day out.

Conclusion: Altogether, the thing of noteworthy is that, a determined effort using the best appropriate food-allergy diagnostic-technics would be doubtlessly, required concerning the acquisition of the most clinically effective/safe patient results and, to develop any effectual/promising therapeutic-approaches for this type allergies owing-to their evident epidemic and worldwide increment in prevalence and morbidity and therefore, it is prudent to go once again critically, through the due pathogenesis to point-out exactly the involved mechanism at each point and/or, know the ropes, to deal more appropriately with food allergy, one of these fine days.

Keywords: FOOD ALLERGY, Pathogenesis, Diagnostic-techniques, Therapeutic-approaches.

Immunomodulatory effects of vitamin D3 on gene expression of MDGF, EGF, and PDGFB in endometriosis

Hadi Mohagheghian[1], Sajjadeh Movahedinia[2], Morteza Samadi[3], Ali-Akbar Delbandi[4]

1. Department of Immunology, Scool of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Pathology, Scool of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, Scool of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
4. Department of Immunology, Scool of Medicine, Iran University of Medical Sciences, Tehran, Iran; Immunology Research Center, Immunology and Infectious Disease Institute, Iran University of Medical Sciences, Tehran, Iran

Background: Endometriosis, an inflammatory disease, is assumed to be associated with an increased production of growth-related cytokines. Based on the emerging immunomodulatory role of vitamin D3 in different inflammatory conditions, this study aimed to examine its modulatory effect on the expression levels of the genes for platelet-derived growth factor-B (PDGFB), monocyte/macrophage-derived growth factor (MDGF, also known as PPBP) and epidermal growth factor (EGF) in peritoneal fluid mononuclear cells (PFMC) in women with and without endometriosis.

Methods: PFMC from 10 women with endometriosis and 10 control participants were treated with vitamin D3. The gene expression levels of PDGFB, MDGF and EGF were measured 6, 24 and 48 h following vitamin D3 administration using real-time PCR.

Results: Gene expression levels of EGF and PDGFB were higher in the PFMC of women with endometriosis than the control group ($P = 0.006$, $P < 0.001$, respectively). Although MDGF expression showed an increase in the endometriosis group compared with non-endometriotic controls, no significant difference was found. Vitamin D3 significantly decreased EGF expression at 6, 24 and 48 h ($p< 0.001$, $p< 0.001$ and $P = 0.007$, respectively), MDGF at 24 and 48 h ($p< 0.001$ and $p= 0.009$, respectively) and PDGFB at 6 h ($p=0.047$) in the endometriosis group. Vitamin D3 treatment had no significant effect on expression of the genes in the PFMC of non-endometriotic women.

Conclusion: The study concluded that PDGFB and EGF gene expression increases in endometriosis, and vitamin D3 could markedly decrease this expression, suggesting its therapeutic potential in endometriosis.

Keywords: Endometriosis, Epidermal growth factor (EGF), Monocyte/macrophage-derived growth factor (MDGF), Platelet-derived growth factor-B (PDGFB), Vitamin D3

N-3 PUFA may inhibits CRC cells immune evasion and metastasis through affecting immunomodulatory genes and related microRNAs

Manouchehr Fadaee[1], Tohid Kazemi[1]

1. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: The immune escape and metastasis of colorectal cancer (CRC) has challenged current immunotherapies. N-3 Poly unsaturated fatty acids (PUFAs), especially Docosahexaenoic acid (DHA), are safe food supplements that control the development of many cancers, including CRC. So, the purpose of this study was to investigate the effect of DHA administration on the expression of PD-L1, CD39, and CD47 and their related miRNAs in CRC.

Methods: Human CRC cell lines, HT-29 and Caco-2, were cultured in RPMI 1640 containing 10% FBS and penicillin at 37 °C in a 5% CO2 incubator. After reaching a concentration above 80%, the cell lines were treated with 100 µM DHA for 24 hours under normoxic and hypoxic conditions. 25 µM Cocl2 was used for applying hypoxic condition. RNA extraction was performed according to the kit protocol and the expression of CD37, CD49, and PD-L1 and miRNAs was detected by real-time quantitative polymerase chain reaction (RT-qPCR). Western blot is used to analyze the expression of PD-L1 protein.

Results: After administration with DHA in normoxic conditions, the expression of PD-L1, CD37, and CD47 was decreased but their controlling miRNAs, mir-424, mir-142, and mir-133a were increased, respectively. Hypoxic conditions reversed the above phenomenon. Western blot analysis showed a reduction in PD-L1 protein expression after DHA treatment.

Conclusion: DHA treatment inhibited significant immunomodulatory genes involved in CRC development. Therefore, the anti-cancer effects of DHA may reduce tumor immune evasion and metastasis. This study showed that DHA can be used as an adjuvant in combination with current treatment methods.

Keywords: Omega-3, Colorectal cancer, tumor invasion, Docosahexaenoic acid

Specialized pro-resolving lipid mediators in cardiovascular diseases: a systematic review

Maryam Haddadi[1], Bahroz Ahmed[1], Alireza Rezaeimanesh[2], Ali Gorgin Karaji[2], Farhad Salari[2]

1. Student Research Committee, Department of immunology, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Department of immunology, School of medicine, Kermanshah University of Medical Sciences, Iran

Background: There is growing interest in using omega-3 derived specialized pro-resolving lipid mediators (SPMs) to prevent cardiovascular disease (CVD) complications. This systematic review aimed to investigate whether omega-3 supplements could impact on SPM profiles and have beneficial effects in patients with CVD.

Methods: Electronic databases, including Pubmed, Scopus, Web of science and clinical trial registry, and CENTRAL, were searched for randomized clinical trials from their inception until July 2022. (PROSPERO CRD42022358453). A quality assessment was performed using Cochrane Collaboration's tool. The primary outcome was a change in the SPMs levels following the intervention, and any health improvement was considered as secondary outcome.

Results: Five studies (out of 683) with patients mean aged >50 were included. In these RCTs, including peripheral arterial disease (PAD) (n=2), coronary arterial disease (CAD) (n=2), and atherosclerosis (n=1), the serum levels of SPMs following oral omega-3 supplementations in patients with CVDs had been assessed. In patients with PAD, there was a significant increase in the level of lipoxin A5 (p=0.05), resolvin E3 (p=0.04), and a significant decrease in the level of lipoxin A4 (0.009). The secondary outcomes were a significant increase in flow-mediated vasodilation (FMD) (p=0.04) and a significant reduction in triglycerides (p<0.001). In patients with CAD, a substantial increase in the level of aspirin-triggered - resolvin D3, resolvin D6, aspirin-triggered-protectin D1, aspirin-triggered - lipoxin B4 (p< 0.001), resolvin E1 (p=0.004), and maresin 1 (p=0.009) had been found. They showed a significant plaque regression and promotion of blood clots phagocytosis; suggested potential improvements in resolution. In addition, no significant differences in SPMs levels were reported in the atherosclerosis trial.

Conclusion: Overall, omega-3 supplementations may result in increasement of serum levels of SPMs and subsequent beneficial effects in patients with CVDs. However, we need further studies with high-quality data to consider SPMs in the clinical setting.

Keywords: specialized pro-resolving lipid mediators (SPMs), cardiovascular disease (CVD), omega-3 supplements

The Association between Vitamin-D Deficiency and COVID-19 Severity

Nima Zafari[1], Zeinab Faghfoori[2]

1. Student Research Committee, Department of Nutrition, School of Nutrition and Food Sciences, Semnan University of Medical Sciences, Semnan, Iran
2. Food Safety Research Center (salt), Department of Nutrition, School of Nutrition and Food Sciences, Semnan University of Medical Sciences, Semnan, Iran

Background: Coronavirus disease 2019 (COVID-19) outbreak has rapidly expanded to a global pandemic and many aspects of its pathogenesis and related clinical consequences are still unclear. Vitamin-D due to its immunomodulatory effect, has been proposed as a factor playing role in the organism response to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The aim of this study was to evaluate the association of vitamin-D deficiency and severity of COVID-19. Clinical features and infection-related risk factors are also briefly discussed.

Methods: PubMed, Google Scholar, Web of Science databases were searched for studies evaluating the association between vitamin-D deficiency and severity of COVID-19 infection.

Results: Hypovitaminosis D is attributed to the increased risk of lung injury and acute respiratory distress syndrome (ARDS) as well as diabetes, cardiovascular events and associated comorbidities, which are the main causes of severe clinical complications in COVID-19 patients. The protective effect of vitamin-D is exerted through multiple mechanisms such as modulation of ACE-2 receptor activity, triggering of innate and adaptive immune responses and reducing the levels of cytokines. Vitamin-D maintains intercellular connections and prevents the penetration of viruses into the depths of a tissue. Vitamin-D helps the immune system by increasing the production of antimicrobial peptides such as cathelicidin and beta-defensin, increasing the phagocytosis of macrophages and stimulating the differentiation of immune cells in the lungs. Also, vitamin-D reduces the excessive secretion of inflammatory cytokines and prevents cytokine storm in the lung and other organs. Regulating the renin-angiotensin system and preventing the sharp increase of angiotensin 2 are other functions of vitamin-D against the corona virus. Vitamin-D deficiency is significantly associated with high mortality, high hospital admission, longer hospital stay and ICU admission. Supplementation can reduce the risk of mortality, severity, ICU admission rate and mechanical ventilation in patients with both mild and severe symptoms.

Conclusion: In general, greater severity of COVID-19 infection can be associated with Vitamin-D deficiency. According to the prevalence of vitamin-D deficiency in populations, supplementation and controlling serum levels of vitamin-D can reduce mortality, especially in high risk individuals. More studies can help to understand the benefit of vitamin D in COVID-19.

Keywords: Coronavirus, SARS-CoV-2, COVID-19, Vitamin D Deficiency, Severity, Mortality

The effect of probiotic supplementation in treating children with atopic dermatitis using the scoring AD (SCORAD) index: a systematic review

Fateme Pakbaz[1], Zohreh Farrokhian[2], Mahdi Karimi[3]

1. Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by relapsing eczematous injuries and severe itching. Probiotic supplementation seems to have promising effects in the prevention and treatment of atopic dermatitis. However, results regarding efficacy have been controversial. The purpose of this study is to evaluate the effect of probiotic supplementation in treating children with atopic dermatitis using the scoring AD (SCORAD) index.

Methods: We searched scientific reports on the PubMed website from February 1, 2003, to August 31, 2022. These studies included data from children with atopic dermatitis treated with probiotics supplementation.

Results: We identified one hundred and three studies on children with atopic dermatitis and probiotics. Fourteen relative publications were eligible for systematic review. Finally, after applying the inclusion and exclusion criteria, 6 clinical trials and randomized clinical trials were found and the following results were obtained from children with atopic dermatitis who received probiotic supplements: In all studies, the severity of AD symptoms as assessed by the Scoring Index for AD (SCORAD) was reduced by at least a 30% reduction in the SCORAD score. The use of topical corticosteroids in probiotic patients decreased by an average of 7.7 grams. In lymphocyte subsets, the percentage of CD4 and the percentage and absolute count of CD25 decreased, and the percentage and absolute count of CD8 increased in the probiotic group. During active treatment, serum eosinophil cationic protein levels decreased (p=0.03). We found no significant changes in producing IL-2, IL-4, and IL-10 cytokines.

Conclusion: Treatment of atopic dermatitis with probiotic supplementation, reduced the SCORAD index and the use of topical steroids in these patients. However, more research is necessary to evaluate the effect of probiotic supplementation on immunity in children with atopic dermatitis.

Keywords: "Atopic dermatitis", "Probiotic", "Children"

Psychoneuroimmunology

A Comparison of Thioredoxin 1 Gene Expression in Schizophrenia Patients before and after Treatment with Risperidone

Morteza Hosseinzadeh[1], Esfandiar Azizi[1], Parviz Vahdatian[1], Amir Adibi[2], Omran Davarinejad[3], Hojat Sayyadi[4]

1. Department of Immunology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran.
2. Department of Child and Adolescent Psychiatry, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran.
3. Department of Psychiatry, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
4. Department of Biostatistics, School of Health, Ilam University of Medical Sciences, Ilam, Iran.

Background: Schizophrenia is a mental disorder characterized by distortions in thinking, perception, emotions, language, self-sense, and behavior. Recent research suggests that Reactive Oxygen Species (ROS) are involved in the pathophysiology of schizophrenia. Studies have also shown the increased plasma and serum levels of the Trx1 molecule in schizophrenia patients. In the present study, the researchers compared the expression levels of Trx1 mRNA in peripheral blood leukocytes of Iranian schizophrenia patients compared to healthy controls.

Methods: First-episode patients (n=35) who met DSM-IV criteria for schizophrenia were recruited from patients referred to psychiatrists in the city of Ilam and Farabi Hospital in Kermanshah. Healthy people were also selected by recruiting people who, according to a psychiatrist, did not have any mental illness. Diagnoses were made for each patient by two independent experienced psychiatrists and confirmed by the Structured Clinical Interview for DSM-IV (SCID). Patients were treated with risperidone for three months and then compared with thirty- five healthy volunteers. Patients were sampled before and after treatment and then by RNA Extraction and DNA synthesis, Trx1 gene expression was performed by real-time PCR method.

Results: Comparison of Trx1 gene expression in PBMCs of schizophrenic patients before and after treatment with the control group showed that the expression of Trx1 gene of the “before” treatment group was significantly increased compared with that of the control group (P= 0.0007). Also, Trx1 gene expression in PBMCs of “before” and “after “groups showed that Trx1 gene expression of “after” group was significantly decreased compared to the “before” group (p=0.014). These results showed that the mean of positive, negative, and general psychopathology was reduced significantly in schizophrenic patients before and after treatment in all three cases (p<0.001).

Conclusion: the expression of TRX in PBMCs of schizophrenic patients decreased after risperidone treatment. This reduction of expression was statistically significant and indicates the possible effect of risperidone on the expression of the TRX gene in PBMCs of these patients and decreased gene expression is associated with reduced symptoms. Confirmation of the achievement of this study requires further research.

Keywords: Schizophrenia, thioredoxin, risperidone

An inclusive study on cytokine gene expression in Parkinson's disease: Advanced analysis using Bayesian regression model

Shadi Moradi[1], Alireza Zamani[1], Mehrdokht Mazdeh[1], Mahdi Ramezani[1], Alireza Komaki[1], Elahe Talebi-Ghane[1], Mohammad Mahdi Eftekharian[1]

1. Hamadan University of Medical Sciences, Hamadan, Iran.

Background: Parkinson's disease (PD) is the second most prevalent neurodegenerative disease throughout the globe its specific pathophysiology is unknown. Researchers believe that inflammation and oxidative stress contribute to PD development. Also, alterations in cytokines production appear to have a key role in the pathogenesis of PD. The aim of the current study was to evaluate gene expression levels of nine cytokines in the peripheral blood of PD patients compared to a healthy control group.

Methods: Real-time PCR was used to analyze cytokines gene expression followed by advanced statistical analysis performed using the Bayesian regression model in R (version 4.1.0) statistical software.

Results: TNF-α, IL-1β, IL-2, IL-4, IFN-γ, IL-17, and IL-6 transcript levels were upregulated in patients compared to healthy controls. However, CXCL8 expression was downregulated in patients compared to controls and IFN-βexpression was not statistically different between the two groups. While we found no significant difference between the groups based on gender and age regarding TNF-α, IL-1β, CXCL8, IL-2, IL-4, IFN-γ, and IFN-β gene expression, IL-6 and IL-17 transcript levels showed significant upregulations in older subjects and in females, respectively. In addition, we found that the interaction effects between gender and group on gene expression levels were not significant. In this way, the subgroup analysis within gender revealed that in each gender, expression levels of TNF-α, IL-2, IL-4, IL-6, IFN-γ, and IL-17 were significantly higher in patients than controls. However, IFN-βexpression level did not show any significant difference between groups and subgroups.

Conclusion: The present study provides evidence of significant alterations in cytokine expression with different patterns and points to immune system dysregulation in PD.

Keywords: Parkinson's disease, Cytokine, Chemokine, Inflammation

Anti-inflammatory and neuroprotective effects of liposomal quercetin via microglia/macrophage polarization dynamics in a Huntington's disease model of male rats

Ali Alishvandi[1], Kamal Abdolmohammadi[2], Ghorbangol Ashabi[3], Kiarash Saleki[4], Farzaneh Faraji Shahrivar[5]

1. Student Research Committee, Iranshahr University of Medical Sciences, Iranshahr, Iran
2. Department of Immunology, School of Medicine, Iranshahr University of Medical Sciences, Iranshahr, Iran
3. Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
4. Student Research Committee, Babol University of Medical Sciences, Babol, Iran/// USERN Office, Babol University of Medical Sciences, Babol, IranDepartment of e-Learning, Virtual School of Medical Education and Management, Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran
5. Department of Physiology, School of Medicine, Iranshahr University of Medical Sciences, Iranshahr, Iran

Background: Huntington's disease (HD) is a neurodegenerative disorder characterized by psychiatric disturbances, dementia and motor dysfunctions. Microglial polarization via inflammation seems to play a pivotal role in HD pathogenesis. The aim of this study was to investigate the polarization of microglia to anti-inflammatory phase for improving microenvironment of striatum of rat brain.

Methods: A total of 18 male Sprague-Dawley rats were randomly divided into 3 equal groups: 1- Sham 2- HD model (Stereotaxic surgery) 3- HD model+liposomal quercetin (30mg/kg). For inducing HD model, the rats were anesthetized, then, a combination of 1ml quinolinic acid and saline (200nmol QA/2µl saline, unilateral) was microinjected into striatum through stereotaxic apparatus. Liposomal quercetin (30mg/kg intraperitoneally) was administered once-daily treatment for two months. Behavioral and locomotor functions were assessed by open field test. After deep anesthesia, the animals were sacrificed and their striatum's were kept in formalin for exerting RT-qPCR gene expression, cresyl violet and immunofluorescence (IF) staining.

Results: The behavioral and locomotor activity of HD rats were significantly decreased, which was very highly significantly improved by Liposomal quercetin ($p<0.001$). Moreover, administration of liposomal quercetin significantly increased the expression of CD206 gene in treatment group compared to the HD model group in the striatum area ($p<0.05$). IF staining also confirmed the upregulation of CD206 protein as an anti-inflammatory marker ($p<0.01$). Although the population of dopaminergic neurons in treatment group had an incrementing trend compared to the HD model, this relationship was not statistically significant ($p>0.05$).

Conclusion: Treatment of HD model rats with liposomal quercetin improved the immune microenvironment of striatum by upregulating anti-inflammatory microglia (CD206: representative marker for M2 phenotype). Further, the effect on behavioral and locomotor functions was favorable. These finding suggest liposomal quercetin as a modulator of microglia polarization as well as a potential treatment for reducing symptoms in patients with HD.

Keywords: Inflammation, Huntington's disease, Liposomes, Quercetin

Assessment of Fractalkine, Fractalkine receptor and plasma level of Neopterin in patients with the major depressive disorder before and after treatment with Sertraline

Samaneh Ebrahimnezhad[1], Hanieh Tarokhian[1]

1. Department of immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

Background: Major depressive disorder (MDD) is a common mental disorder that inflammation is one of the most effective factors in the pathogenesis of this disease. Fractalkine is a chemokine that is involved in controlling inflammation in the brain through its receptor. Neopterin is a purine metabolite whose production increases following the inflammation that is associated with the production of neurotransmitters such as serotonin. In this study we aim to investigate the alteration of this inflammatory variables following stressful condition.

Methods: Seventeen patients with MDD and seventeen healthy subjects were included in the study as a control group. Patients were treated with Sertraline for 2 months. Blood samples were obtained from healthy and patient individuals, then neopterin plasma concentration were measured by ELISA, and CX3CL1 and CX3CR1 genes expression levels were evaluated using RT-qPCR.

Results: The gene expression of CX3CL1 and CX3CR1 in the baseline were not significantly different from the control group. After treatment, the expression level of CX3CL1 increased significantly compared to baseline ($p= 0.002$), but no significant difference was observed with the control group. After the treatment the gene expression level of CX3CR1, increased significantly compared to pre-treatment ($p= 0.03$) and the control group ($p= 0.04$). The plasma level of neopterin in patients was higher than in the control group, which decreased after treatment compared to the baseline and the control group, but these differences were not statistically significant. After treatment, the average score of the PHQ-9 decreased significantly compared to baseline ($p<0.0002$), but the difference between patients and control groups was still significant ($p<0.0002$).

Conclusion: A significant increase in the expression of CX3CL1 and CX3CR1 genes, along with a significant decrease in the score of the PHQ-9 following Sertraline treatment, demonstrated the efficacy of this kind of antidepressive therapy on controlling inflammation and maintaining homeostasis in the brain.

Keywords: Major depressive disorder, MDD, CX3CL1, CX3CR1, Neopterin, Sertraline

Assessment of IL-21 and IL-22 in major depressive disorder follow-up sertraline treatment

Reihaneh Khaleghi Moghadam[1], Hanieh Tarokhian[1]

1. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

Background: According to a recent preclinical framework, inflammation has an essential role in the pathogenesis of major depression disorder (MDD). Cytokines are the main contributors to inflammation, which are secreted by various adaptive and innate immune cells. Several studies examined a wide variety of inflammatory cytokines in MDD and indicated that there is chronic inflammation during depression. However, few studies have investigated the role of cytokines secreted by TH17, cytotoxic CD8+T, and NK cells, such as IL-21 and IL-22. Therefore, the inflammatory profile of depression raised the hypothesis that these cells may promote the depression index. Herein, we assess the serum level of IL-21 and IL-22 cytokines in MDD patients before and after treatment with sertraline.

Methods: We measured IL-21 and IL-22 cytokine serum levels by enzyme-linked immunosorbent assay (ELISA) in 17 patients with MDD before and 8 weeks after sertraline treatment and also in 17 healthy controls.

Results: Compared to the healthy control group, the serum level of IL-22 was significantly higher in patients with MDD (p=0.022). However, the serum level of IL-21 in two groups of patients compared to the healthy control was not significant (p=0.064). There was no significant difference in the levels of these two cytokines after 8 weeks of sertraline treatment compared to baseline (p=0.19, p=0.053). There was a significant correlation between IL-21 and IL-22 before and after treatment with sertraline, and among these two, IL-22 before treatment had a significant correlation with the depression index in MDD patients.

Conclusion: Considering previous studies, IL-21 and IL-22 have different functions in inducing inflammation. Moreover, consistent with the inflammatory hypothesis of depression our results showed that IL-22 significantly increased in MDD patients. However, investigating the role of IL-21 in the pathogenesis of this disease still needs further investigation.

Keywords: IL-21, IL-22, MDD, cytokine

Autoantibodies against central nervous system antigens and the serum levels of IL-32 in patients with schizophrenia

Fatemeh keshavarz[1], fatemeh Azadegan-Dehkordi[2], Nader bagheri[3]

1. Department of Immunology, Shahid Beheshti University of medical scieneces, Tehran, Iran
2. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
3. Department of Microbiology and Immunology, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

Background: Immune system problems may have an impact on the development of schizophrenia, which is a neurological system disease. Among the immunological mechanisms implicated in neurological illnesses include activation of microglia, proinflammatory cytokines, disruption of the blood-brain barrier (BBB) due to inflammation, activation of autoreactive B lymphocytes, and therefore the creation of autoantibodies against system antigens. Interleukin -32 (IL-32) is a proinflammatory cytokine that is essential in activating innate and adaptive immune responses. This study aimed to measure the serum level of IL-32 as well as the frequency of autoantibody positivity against several nervous system antigens in patients with schizophrenia.

Methods: This study was conducted on 40 patients with schizophrenia and 40 healthy individuals in the control group. Serum IL-32 levels were measured by ELISA. The frequency of autoantibodies against Zic4, ITPR1, CARP, GAD, Recoverin, Titin, and Ganglioside antigens were measured by the indirect immunofluorescence method.

Results: Serum IL-32 levels in patients with schizophrenia were significantly higher compared to the control group. The frequency of autoantibodies against GAD in patients with schizophrenia was significantly higher than in the control group. Autoantibodies were positive in 8 patients for GAD antigen. Autoantibodies were also positive in 2 patients for CV2 and CARP. Negative results were reported for other antigens.

Conclusion: Our findings suggest that elevated serum IL-32 levels and autoantibodies against GAD and RI antigens may be a reflection of immune system dysregulation in patients with schizophrenia.

Keywords: Schizophrenia; Microglia; Autoantibodies; IL-32

CD Protein Markers as Potential Therapeutic Targets in a Mouse Stroke Model

Mohammad Matin Nourikhani[1], Amir Gholamzad[1], Omid Moeini[1], Seyed Armit Hosseini[1], Mehrdad Gholamzad[1]

1. Department of Laboratory Medicine, Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

Background: Stroke is a leading cause of death and disability worldwide. CD proteins are involved in various cellular processes, including cell adhesion, migration, and proliferation, and their dysregulation has been implicated in the pathophysiology of stroke. However, their potential as therapeutic targets in stroke has not been extensively explored.

Methods: A mouse model of stroke was induced by middle cerebral artery occlusion (MCAO). The expression of CD proteins, including CD44, CD47, and CD73, was evaluated in the brains of stroke-induced mice using immunohistochemical and western blot analyses. The effects of CD protein blockade on stroke outcomes were also assessed.

Results: CD44 and CD47 expression were significantly increased in the brain tissues of stroke-induced mice compared to control mice. In contrast, CD73 expression was reduced in the ischemic penumbra of the stroke-injured brain. CD44 and CD47 blockade using monoclonal antibodies led to improved functional outcomes, reduced inflammation, and decreased infarct size in stroke mice.

Conclusion: CD proteins, particularly CD44 and CD47, play important roles in the pathophysiology of stroke and could represent promising therapeutic targets for stroke treatment. Their blockade using monoclonal antibodies could have beneficial effects on stroke outcomes, including reduced inflammation and infarct size. Further studies are warranted to explore the full extent of CD protein dysregulation in stroke and evaluate their potential as therapeutic targets.

Keywords: CD Marker, Stroke, Neuroimmunology

Evaluation the effect of Lactobacillus delbrukii and Lactobacillus rhamnosus probiotics as complementary therapy with levodopa in modulating behavior and immune response in Parkinson's rat model

Narges abdollahi[1], Seyed-Alireza Esmaeili[1], Mahmoud Mahmoudi[1]

1. Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Parkinson's disease (PD) is the second most common progressive neurological disease worldwide. The main cause of the disease is unknown, systemic inflammation plays a major role in the initiation and progression of the disease. The disease has motor and non-motor symptoms. Currently, levodopa administration as a treatment strategy is based on control of the motor symptoms and long-term use of drug is associated with some side effects. Therefore, considering the insufficient effectiveness of the existing drug, the need for new medicines is felt. According to recent studies, tolerogenic probiotic supplements reduce inflammatory cytokines and oxidative stress while increasing anti-inflammatory cytokines and can effectively modulate the disease. Therefore, current study was conducted to evaluate the effect of probiotics as therapeutic supplements with levodopa on Parkinson's rat model.

Methods: PD in male Wistar rats was induced by 6-hydroxydopamine and confirmed by the apomorphine test. Prophylaxis and treatment of rats with a mixture of probiotics Lactobacillus delbrueckii and Lactobacillus rhamnosus and also treatment with a mixture of probiotics along with the administration of Levodopa drug were carried out. Finally, behavioral tests, periodic acid shift staining of intestinal tissue, oxidative tests, inflammatory and non-inflammatory cytokines were evaluated by the Real-time PCR method.

Results: the study is in process and the data will be presented in congress.

Conclusion: According to the anti-inflammatory effects of mentioned probiotics, it is expected that the level of inflammation and oxidative stress will be reduced by our probiotics and be a good option to use as a complement drug.

Keywords: Parkinson's disease, Lactobacillus delbrukii, Lactobacillus rhamnosus, inflammation and control disease

Increased IL1β& IL1RAcP expression levels and increased IL1B serum levels among IL 1 family of cytokines in patients with Parkinson's disease

Mohammad Mahdi Eftekharian[1], Negin Jafariaghdam[1], Majid Khoshmirsafa[2], Alireza Zamani[1], Faeze Shahba[2], Elahe Talebi-Ghane[3], Shadi Moradi[1]

1. Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2. Department of Immunology, School of medicine, Iran University of Medical Sciences, Tehran, Iran
3. Modeling of Noncommunicable Diseases Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

Background: Parkinson's disease (PD), the second-most common neurodegenerative disorder without any recognized etiology, is known to be affected by oxidative stress and changes in the levels of inflammatory cytokines. This study's objective was to determine the expression levels of IL1 receptor accessory protein (IL1RAcP), IL1β IL1α IL33, and IL36 genes in the peripheral blood mononuclear cells (PBMCs) of patients with PD compared with healthy control group (HCs).

Methods: The quantitative real-time PCR (qRT-PCR) method for assessing gene expression levels and the enzyme-linked immunosorbent assay (ELISA) method for evaluating serum levels were employed in this study, proceeded by advanced statistical analyses utilizing the Bayesian regression model developed in R software (version 4.1.0).

Results: Patients with PD had higher expression levels of IL1RAcP and IL1βgenes, but lower levels of IL1α IL33, and IL36 gene expression levels compared with HCs. Based on age, there was no significant difference between the groups; however, based on gender, IL33 transcript levels were significantly downregulated in males than in females. Additionally, serum levels of IL1βin patients with PD were found to be higher compared with HCs group. The subgroup analysis within gender showed that females are more responsible for higher serum levels of IL1βand men are more responsible for lower expression levels of IL1α IL33, and IL36.

Conclusion: Substantial variations in the expression levels of IL1RAcP, IL1β IL1α IL33, and IL36, as well as IL1βserum levels with diverse patterns in findings of present study, imply the involvement of dysregulation of these factors and the immune system in PD.

Keywords: Parkinson's disease, PD, IL1RAcP, IL1β IL1α IL33, IL36, Neuroinflammation, Neuroimmunology,

Investigating stress-induced atherosclerosis in rats with an early life stress history

Motahare Hatami[1], Nayere Askari[2]

1. Department of Cardiology, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran. .
2. Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran

Background: Atherosclerosis is a progressive inflammatory disease that is recognized as the main cause of cardiovascular disease (CVD). An accumulating body of evidence indicates that psychological stress can predict both the onset and progression of CVD. It is clear that the sensitivity and vulnerability of living organisms under stress are different. The mechanism for these variations is not well-defined. The main purpose of this study was to investigate the effects of neonatal stress on the development of atherosclerosis in adult rats in the face of stressful events, and the role of HPA and MMP9 in the course.

Methods: Forty-two rats were assigned to six groups (n=7), control, neonatal stress, social stress, unpredictable chronic stress, neonatal stress + social stress, and neonatal stress + unpredictable chronic stress. Male offspring of Wistar rats were subjected to 3 h of daily maternal deprivation (MD) during postnatal days 1-14. In adulthood, rats were subjected to social stress and chronic unpredictable stress. By histological examination, the aorta of the studied animals was compared in terms of atherosclerotic plaque formation. Serum corticosterone levels were measured by the ELISA method. Gene expression was determined by qPCR.

Results: Neonatal stress groups had higher corticosterone levels. A significant decrease was observed in the expression of MR and GR (glucocorticoid receptor) genes in neonatal stressed rats. Also, a significant increase in MMP9 expression was observed in both groups. Histological studies showed progression in atherosclerosis plaque formation, especially in animals under neonatal stress with exposure to social stress.

Conclusion: Exposure to adult stress in the maternal deprivation group leads to more severe stress-induced responses in the development of aortic atherosclerotic lesions, related gene expression, and serum corticosterone levels. According to the results, it is suggested that exposure to early life stress causes more sensitivity to stressors in adults.

Keywords: atherosclerosis, early life stress, MMP9, HPA

Neonatal stress-induced impairments in learning and memory are related to changes in TLR2/4 in selected rat brain regions (hippocampus, prefrontal cortex).

Manijeh Dogani[1], nayere askari[2], Mohammad-Reza Vaez-Mahdavi[3], Tooba Ghazanfari[3]

1. Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran. 2. Immunoregulation Research Center, Shahed University, Tehran, Iran
3. Immunoregulation Research Center, Shahed University, Tehran, Iran

Background: The fetal period is one of the most sensitive and critical periods of life, and prenatal stress can have significant effects on mental health and human development across the lifespan. Although chronic stress impairs brain cognitive functioning, the mechanism remains unclear. According to previous studies, chronic mild stress increases TLR expression in the prefrontal cortex of male rats and also activates innate immune Toll-like receptors (TLRs). We hypothesized that maternal stress could induce changes in TLR2/4 gene expression, so may be involved in the impairment of spatial memory.

Methods: Pregnant female rats were exposed to restraint stress and in adulthood, male offspring received chronic unpredictable stress (CUS), and social instability stress for three weeks. Passive avoidance, active avoidance, and spatial learning and memory using the shuttle box and Morris Water Maze tasks were investigated, respectively. Expression of TLR2/4 genes in rats' hippocampus and prefrontal cortex was determined by qPCR.

Results: The prenatal stress group showed impairments in learning and memory function. These disorders are aggravated in prenatally stressed rats exposed to social stress. TLR2 and TLR4 mRNA levels in the prefrontal and hippocampal tissues of the prenatal stress group animals were significantly higher than in the control group. In rats with a background of prenatal stress, exposure to adult stress causes a greater increase in gene expression. The highest expression of TLR4 mRNA is seen in rats with a background of maternal stress and receiving social stress in puberty.

Conclusion: These results indicate that exposure to prenatal stress causes permanent deficits in long-term memory formation and/or retrieval, which continue into adulthood and exacerbates chronic social stress in adulthood. Stress leads to TLR2 and TLR4 upregulation. TLR2 and TLR4 have been identified as critical mediators of neuroinflammation, which leads to neuronal changes and anxiety.

Keywords: Prenatal Stress, Social Stress, Learning and Memory, Toll-like receptors.

Neuropsychological function is related to irritable bowel syndrome in women with premenstrual syndrome and dysmenorrhea

Malaksima Ayadilord[1], Mohsen Naseri[2]

1. Department of Medical Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.

Background: There is increasing evidence demonstrating the co-occurrence of primary dysmenorrhea (PD), premenstrual syndrome (PMS), and irritable bowel syndrome (IBS) in women. This study aimed to investigate whether women who have symptoms of IBS in addition to PD and PMS also report more severe or frequent menstruation-associated symptoms and psychological complications compared to women with PD and PMS alone.

Methods: The study group included 182 female University students aged 18–25 years. IBS was diagnosed using the Rome III criteria. The severity of PMS and PD was determined using a 10-point visual analog scale and PSST (Premenstrual Syndrome Screening Tool), respectively. Neuropsychological functions including cognitive function, depression score, anxiety score, stress, insomnia, daytime sleepiness, quality of life and personality were assessed using standard questionnaires.

Results: Of the 182 young females, 31 (17.0%) had IBS. Average days of bleeding during the menstrual cycle and mean pain severity on the PSST scale were significantly greater in the group with IBS compared to the non-IBS group ($p<0.01$). The non-IBS individuals scored more favorably than the women with IBS with respect to severity of depression, insomnia, daytime sleepiness ($p< 0.05$). The PSST scores were significantly correlated with scores for depression ($r = 0.29$; $p<0.001$), anxiety ($r=0.28$; $p< 0.001$), stress ($r=0.32$; $p< 0.001$), insomnia ($r = 0.34$; $p < 0.001$) and daytime sleepiness ($r = 0.31$; $p< 0.001$); while, they were negatively correlated with cognitive abilities ($r = -0.20$; $p = 0.006$) and quality of life ($r=- 0.42$; $p< 0.001$). Linear regression analysis showed that the PSST scores were possibly significant factors in determining the scores for depression, anxiety, stress, quality of life, insomnia and daytime sleepiness ($p< 0.05$).

Conclusion: IBS is related to psychological comorbidities, in particular depression, sleep problems and menstrual-associated disorders. IBS may exacerbate the features of PMS which should be taken into account in the management of PMS.

Keywords: Cognitive abilities, Anxiety, Depression, Insomnia, Quality of life

Repeated systemic lipopolysaccharide injection induced depressive-like behavior but not anxiety-like behavior and spatial memory impairment in rats.

Hossein Salmani[1], Ahmad Golkar[2], Hedyeh Askarpour[1], Mohammad Mehdi Jafari[2]

1. School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran
2. Student Research Committee, Jiroft University of Medical Sciences, Jiroft, Iran

Background: The critical role of systemic inflammation in the pathophysiology of brain diseases has been frequently reported. Recently, it has been proposed that brain may become more sensitive to systemic inflammation if microglial cells are already primed or sensitized. Microglial priming has been demonstrated in aging, traumatic brain injury, neurodegenerative disease and systemic inflammation models. The aim of this study was to investigate the behavioral consequences of chronic systemic inflammation on rats that previously undergone a single and proportionally high dose lipopolysaccharide (LPS) challenge.

Methods: Rats were intraperitoneally (i.p) injected with 1 mg/kg LPS or saline. Two weeks after the first LPS challenge rats received repeated LPS (0.5 mg/kg; i.p.) or saline injection for 7 continues days. After the last LPS injection the behavioral tests were carried out within two following weeks. Morris water maze (MWM), (for the evaluation of spatial memory), elevated plus maze (EPM; for assessing anxiety-like behaviors), and forced swimming test (FST) and sucrose preference test (SPT) (for assessing depressive-like behaviors) were used. Data were analyzed using repeated measure ANOVA and independent t-test.

Results: Repeated systemic inflammation increased immobility time, and reduced climbing time in FST ($p<0.05$), and reduced sucrose consumption in SPT test ($p>0.05$) indicating long-term depressive-like behaviors. However, there were no significant differences in escape latency and time spent in the target quadrant in the MWM test between the control and LPS group ($p> 0.05$). LPS injection also did not significantly changed rats' performance in the EPM test ($p> 0.05$) when tested 10 days after the last LPS injection.

Conclusion: Results indicate that chronic systemic inflammation has deleterious and long-lasting consequences on brain function and particularly depressive-like behaviors can persist even after resolving of inflammation.

Keywords: Systemic inflammation, Depression, Neuroinflammation

The Possible Protective Roles of C. spinosa against neuro-inflammation and neuro-toxicities of Acrylamide

Arezoo Rajabian[1], Fahimeh Lavi Arab[1]

1. Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Capparis spinosa (family: Capparaeae) has been traditionally used as a medicine to relieve various ailments including rheumatism, pain and inflammatory diseases. Acrylamide (Acr) is an environmental pollutant with well-demonstrated neurotoxic and neurodegenerative effects. The present study aimed to evaluate whether C. spinosa extract protects against Acr-induced neuro-inflammation.

Methods: Rats were divided into five groups including negative control, Acr-intoxicated group (10 mg/kg/day), C. spinosa treated groups (100 and 300 mg/kg/day). After treatments, lipid peroxidation in brain tissue was measured. Furthermore, mRNA expression of neuro-inflammatory cytokines was assessed using quantitative real-time polymerase chain reaction (qRT-PCR) in brain tissue.

Results: Acr promoted the brain inflammatory responses via increasing the levels of IL-6, IL-1β and TNF-αwhen compared with the control normal rats (p<0.001). However, C. spinosa markedly attenuated neuro-inflammation as well as brain damage as indicated by the decreased lipid peroxidation when compared with the Acr-intoxicated group (p<0.01 and p<0.001).

Conclusion: The current investigation confirmed the neuroprotective capacity of C. spinosa against Acr-induced brain injury, and our results indicate that anti-inflammatory properties of C. spinosa may play an important role in the C. spinosa-mediated protective effects against Acr-triggered neurotoxicity.

Keywords: Neuo-inflammation, Acrylamide, Cytokine, TNF-α

Reproductive Immunology

A drug delivery system using Rosmarinic acid loaded to exosome improve endometritis by blocking the TLR4-NLRP3 signaling pathway

Morteza Taravat[1], Reza Asadpour[1]

1. Department of clinical Science School of Veterinary Medicine University of Tabriz

Background: The TLR4-NLRP3 signaling pathway plays an essential role in the development of inflammation and especially endometritis. Rosmarinic acid (RA) can have potent anti-inflammatory effects in the drug-loading system. The purpose of this was to evaluate the anti-inflammatory effects of RA loaded to exosomes (RLE) on lipopolysaccharide (LPS)-induced endometritis in mice.

Methods: RA was loaded into serum-derived exosomes, using sonication methods. Animals in the treatment groups were subjected to uterine horn injection of RA, exosome, RA combination with exosome (R+E) and RA loaded to exosome (RLE) in uterine horn by two dosages in each group (5 and 10 mg/kg of RA or exosome),24 hours after induced endometritis. Histopathological analysis, immunohistochemistry, and qPCR were used to determine whether the treatment groups were adequate in controlling inflammation.

Results: The results showed that treatment groups, and mainly RLE10 and R10+E10 groups, could modulate pathological changes, and significantly reduce the gene and protein expression of Toll-like receptor4 (TLR4), Nod-like receptor3 (NLRP3), inflammatory cytokines such asInterlukin-1β (IL-1β) Intelukin-18(IL-1β) IL-18, and Tumor necrosis factor-α(TNF-α), and lastly, Gasdermin-D as a pyroptosis factor.

Conclusion: In conclusion, RA loaded and in combination with exosomes in a dosage of 10 mg/kg (RLE10 and R10+E10) improved endometritis in mice through a suppressing TLR4-NLRP3 signaling pathway.

Keywords: Endometritis, NLRP3, qPCR, Immunohistochemistry

Adoptive regulatory T cell therapy affects fetal resorption through changes in immune cells composition of the uterine and placenta of pregnant abortion-prone mice

Farah Idali[1], Hannaneh Golshahi[2], Leila Katouzian[3], Farzaneh Notash-Haghighat[4], Sanaz Rezaii-nia[1], Mahmood Jeddi-Tehrani[4]

1. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.
2. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3. Reproductive Immunology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
4. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

Background: One of the most notable immunological regulations is the mother's immune tolerance to fetal semi-allografts. Interactions among immune cells, decidual stromal cells, and trophoblasts represent an efficient system of cellular relationships in the maternal-fetal interface.

Methods: In this study, the effect of regulatory T cell (Treg) therapy on the composition of the local leucocyte population of mice prone to spontaneous abortions was investigated. Regulatory T cells were induced in vitro (iTregs) for 96 hours in the presence of 17β-oestradiol (E2), progesterone (P4), and transforming growth factor (TGF)-β1. On days 1-4 of pregnancy, iTregs were injected intravenously into DBA/2-mated pregnant CBA/J female mice (abortion prone). On day 14 of pregnancy, mice were sacrificed and decidua and placenta tissues were removed for cellular composition analysis.

Results: Fetal resorption was significantly decreased in all treated groups. Abortion-prone mice (PBS treated) showed significantly increased resorption rate, increased number of CD3+CD8+ *($p<0.05$)*, lower IDO+ *($p<0.05$)*, and increased uterine natural killer cells (uNK) cell numbers *($p<0.001$)* in the uterus, as well increased NK cells in placenta *($p<0.05$)* in comparison to normal pregnancy group (C+). Pathology staining showed a lower number of uNK cells in the uteri from TGF-β1 *($p<0.05$)*, E2- iTregs *($p<0.001$)*, and P4-iTregs *($p<0.05$)* than in the PBS-treated abortion-prone mice. In the placenta, we found significantly lower numbers of NK cells in TGF-β1-, E2- and P4-iTregs- treated groups than in the PBS-treated group *($p<0.05$, $p<0.05$*, and *$p<0.01$*, respectively).

Conclusion: We propose that modulation of uterine NK cell activity through immunotherapy using Treg cells should be given more attention as an immunological strategy in the treatment of recurrent miscarriage.AcknowledgmentsThis work was supported by a grant (973377) from National Institute for Medical Research Development (NIMAD) and a grant (96-037) from Avicenna Research Institute.

Keywords: Abortion, Natural killer cells, Regulatory T cells.

Analysis of the frequency of MDSCs in an Abortion-Prone Murine Model

Mohammad Ali-Hassanzadeh[1], Mahtab Fasihi[2], Ahmad Golkar[2], Mohammad Dalfardi[2], Reyhaneh Farrokhi[2], Moslem Ahmadi[3], Behrouz Gharesi-Fard[3]

1. Department of Immunology, School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran
2. Student Research Committee, Jiroft University of Medical Sciences, Jiroft, Iran
3. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Recurrent spontaneous abortion (RSA) is a serious pregnancy disorder defined as three or more consecutive pregnancy losses before the 20th week of pregnancy and affects 1–5% of couples trying to conceive. One of the main reasons for RSA is the dysfunction of immune response and immune cells at the feto-maternal interface.

Methods: We evaluated the frequency of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) in the bone marrow and uteroplacental tissue of mice on three gestational days (gd9.5, gd13.5, and gd17.5) using the flow cytometry technic. In this study, we used CBA/J × DBA/2 mice as abortion prone group and CBA/J × Balb/C mice as the normal pregnancy group.

Results: The frequency of G-MDSCs in the bone marrow of abortion-prone mice was decreased at gd9.5 ($p = 0.026$). In uteroplacental tissue, the frequency of G-MDSCs was lower at gd 9.5 and gd13.5 ($p = 0.001$, $p = 0.029$, respectively).

Conclousion: The decreased frequency of G-MDSCs in the bone marrow and at the feto-maternal interface contributes to pregnancy complications.

Keywords: Myeloid-derived suppressor cells, Treg cells, Pregnancy, Uterus, Abortion-prone mode

Association between rs1049174 NKG2D gene polymorphism and idiopathic recurrent spontaneous abortion in Iranian women: a case-control study

Amir Abdian Asl[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Natural killer group 2, member D (NKG2D) is one of the best-known activating receptors of NK cells, which recognizes its ligand on altered or stressed cells and activates NK cells to kill them. Given that few studies have investigated the association between NKG2D gene polymorphism and RSA risk, the current study was carried out to explore the correlation between NKG2D polymorphism (rs1049174 G/C) and RSA risk in Iranian women.

Methods: In this study, the single nucleotide polymorphism of the NKG2D gene for rs1049174 mutation was compared in 140 women with recurrent spontaneous abortion (RSA) and 175 control women with at least one successful pregnancy and without any known pregnancy loss.

Results: The findings just revealed that GG genotype and G allele were significantly higher in the case group compared with the control group *(p<0.001)*. Our results regarding decreased risk of RSA in C allele (OR ¼ 0.438; 95%CI ¼ 0.310–0.619; *p<0.001*), and GC genotype (OR ¼ 0.492; 95%CI ¼ 0.214–0.574; *p<0.001*) compared with G allele and GG genotype respectively.

Conclusion: The results suggest a protective function of C allele because it significantly decreased the risk of RSA compared to G allele. It improves inhibition of NK cells and probably participates in maintaining pregnancy in fertile controls; whereas G allele is related to a slight inhibition of NK cells, probably leading to increased effectiveness of NK activation and undesirable inflammation, which consequently causes fetal rejectionThis study demonstrated a significant association between NKG2D gene polymorphism (rs1049174 G/C) and the risk of RSA in Iranian women.

Keywords: NKG2D, Polymorphism, Recurrent Spontaneous Abortion, NK Cell

CD155 and TIGIT as Immune-Modulator Receptor and Ligand on CD4+ T cells in Preeclampsia Patients

Amin Kamrani[1], Mohammad Sadegh Soltani-Zangbar[1], Shabnam Babaei[1], Forough Chakeri-Khiavi[2], Aref Chakeri-Khiavi[3], Shahla Danaii[4], Mehdi Yousefi[1]

1. Immunology Department, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2 Stem Cells Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Guilan University of Medical Sciences, Rasht, Iran
4. Gynecology Department, Eastern Azerbaijan ACECR ART Center, Eastern Azerbaijan Branch of ACECR, Tabriz, Iran

Background: One of the main characteristics of preeclampsia (PE) is systemic inflammation. Immunoreceptor tyrosine-based inhibitory motif (ITIM) domain and (TIGIT)/CD155T-cell immunoglobulin pathway, have necessary roles in the development of normal pregnancy by promoting regulatory T (Treg) cells. In this study, we aimed to assess the frequency of CD155 and TIGIT expressing TCD4+ cells in both healthy pregnant women and PE.

Methods: In this work, 52 PE patients and 59 healthy, were designated to obtain their venous blood. The isolation of peripheral blood mononuclear cells (PBMCs) was performed from the blood samples, and PBMCs were then cultured in the RPMI-1640 medium. The flow cytometry technique assessed the percentage of TIGIT+ and CD155+ CD4+ cells in PBMCs. Cell culture supernatants were used to determine the secretory levels of some factors, using enzyme-linked immunosorbent assay technique in pregnant women with or without PE both before and after blocking TIGIT and CD155. The mRNA expression of TIGIT, Foxp3, SHP-1, CD155, TGF-β, IL-17, IL-10, and TNF-α, and IL-1β was also evaluated by qRT-PCR in PBMCs.

Results: The results revealed a remarkable decrease in the frequency of CD155+ CD4+ and T TIGIT+ CD4+ cells in PE women, in comparison with the control group. Our data demonstrated decreased protein and mRNA levels of IL-10, CD155, TIGIT, FOXP3, and SHP-1 in PE patients. Moreover, significant enhancement in the levels of IL-17, TNF-α, and IL-1β was approved in PE patients, as compared with the controls. But, blocking CD155 and TIGIT could decrease anti-inflammatory cytokines and increase these inflammatory cytokines.

Conclusion: The results showed that there existed dysregulation between inflammatory and anti-inflammatory profiles, with an inflammatory status polarization, in PE patients. Also, TIGIT/CD155 activated ITIM and demonstrated a positive effect on immune regulation, approving the potential therapeutic value of the TIGIT/CD155 pathway in PE treatment.

Keywords: Preeclampsia, TIGIT, CD155, cytokine

Comparative Study of the Immunomodulatory Effects of Two Accepted Immunotherapies on NKT Subsets in Women with Recurrent Spontaneous Abortion

Fatemeh Rezayat[1], Nafiseh Esmaeil[1], Roya Sherkat[2], Abbas Rezaei[1]

1. Department of Immunology School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Immunodeficiency Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Natural killer T (NKT) cells play a critical role in pregnancy failures, including recurrent spontaneous abortion (RSA). Different approaches are used for these disorders due to their effects on maternal immunomodulation. In the present study, we compared the effects of two typical immunotherapies (lymphocyte immunotherapy (LIT) and low-dose prednisolone) on CD3+CD56+CD16+ and CD3+CD56+CD8+ cells as two distinct subsets of NKT cells in Women with RSA.

Methods: Fifty women in the group of low-dose prednisolone therapy, fifty women in the lymphocyte immunotherapy (LIT) group, and five women without any treatment as the control group were included in the study. NKT cell subsets were assessed using flow cytometry. Also, the level of IFN-γ was measured by the ELISA.

Results: In contrast to the LIT group, the administration of prednisolone increased CD3+CD8+CD56+ NKT cells *($p<0.0001$)*, which is helpful for pregnancy. The effect of the investigated treatment approaches on the population of peripheral CD3+CD56+CD16+ NKT cells of women with RSA was not adequately significant. The same situation was also observed regarding the serum level of IFN-γ.

Conclusion: The lower capability of LIT in changing the population of NKT cells compared to prednisolone therapy may be due to its mechanism of action, which is related to the production of blocking antibodies. These treatment approaches had different effects on NKT cells, indicating that NKT cell population and function can be affected using LIT and prednisolone therapy distinctly. In addition, prednisolone therapy and LIT in women with normal serum levels of IFN-γ have no harmful effects in changing the production of this critical cytokine.

Keywords: Natural killer (NKT) cells, recurrent spontaneous abortion (RSA), prednisolone, lymphocyte immunotherapy (LIT)

Comparison of the Morphology and Regulatory Function of Amniotic Epithelial Cells (AECs) in Women with a History of Recurrent Spontaneous Abortion to Healthy Women

Fatemeh Rezayat[1], Nafiseh Esmaeil[1], Abbas Rezaei[1]

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Recurrent spontaneous abortion (RSA) is a common gynecological disorder in young women caused by the loss of feto-maternal immunotolerance. The amniotic membrane is a crucial protective layer of the fetus involved in immunotolerance. In this study, we investigated the immunomodulatory status of the amniotic membrane at the end of a successful pregnancy to determine whether the health of the membrane in women with a history of RSA is similar to healthy women.

Methods: Fifteen amniotic membranes were separated after elective cesarean delivery from the placenta of women with a history of more than three miscarriages in the first trimester of pregnancy. Fifteen Amniotic membranes were also obtained from the placenta of healthy women. The membranes were investigated microscopically for the number and morphology of amniotic epithelial cells (AECs). Also, the production of anti-inflammatory cytokines by AECs in co-culture with allogeneic NK cells was evaluated by ELISA.

Results: In both study groups, amniotic membranes were similar in AECs purification efficiency, cell integrity, cell morphology, and survival rate in culture conditions in vitro. After the co-culture of AECs with NK cells, no significant difference was observed in the secretion of TGF-β and IL-10 cytokines between the RSA and healthy groups *($P>0.05$)*.

Conclusion: In this study, we did not observe any significant difference between women with RSA and healthy women in the morphology of AECs and the production of TGF-β and IL-10 cytokines in the co-culture with NK cells. Therefore, it can be concluded that in successful cases of pregnancy in women with RSA, the amniotic membrane is healthy enough to protect the fetus.

Keywords: Recurrent spontaneous abortion, amniotic epithelial cell, immunomodulation

Dendritic cells modulation by mesenchymal stem cells promises a protective microenvironment at the feto-maternal interface: improved outcome of pregnancy in abortion-prone mice

Maryam Eskandarian[1], Seyed Mohammad Moazzeni[1]

1. Tarbiat Modares University, Tehran, Iran.

Background: Recurrent spontaneous abortion is one of the most common complications of pregnancy. A major fraction of RSA is closely related to disorders of the maternal immune system, especially malfunction of dendritic cells (DCs) as the main immunoregulatory cells that play a crucial role in the induction of tolerance at the feto-maternal interface. MSCs have been shown to exert immunomodulatory effects on immune cells, especially dendritic cells.

Methods: Considering the probable role of dendritic cells in RSA etiology and the immunomodulatory properties of MSC on dendritic cells, we undertook the current study to investigate whether MSCs are capable to modulate the pattern of maternal immune response via the induction of functional changes in decidual DCs, and finally improves the fetal survival and reduces the rate of abortion. For this issue, adipose-derived mesenchymal stem cells (AD-MSCs) were intraperitoneally administered to abortion-prone pregnant mice (CBA/J ×DBA/2) on the fourth day of gestation. In the control group, PBS was injected. On day 14.5 of gestation, the number, phenotype, and maturation state of decidual dendritic cells were analyzed using flow cytometry.

Results: The abortion rate was significantly decreased following mesenchymal cell therapy of abortion-prone mice. Also the expression of MHC-II, CD86, and CD40 markers, as costimulatory molecules and maturation markers of DCs, remarkably decreased on decidual DCs in the MSC-treated group. In contrast, CD11b significantly increased in this group compared to non-treated mice. MSCs can change the microenvironment of decidua and modulate the DCs' phenotype and functions through the secretion of various components or direct cell-cell contact.

Conclusion: Considering the mutual role of DCs in the induction of tolerance to fetal antigens and vascular modulation during implantation, it seems that MSCs decrease the abortion rate by modulating the DCs' functions as one of its immune protective mechanisms.

Keywords: abortion, dendritic cell, tolerance, Mesenchymal stem cell

Differential effects of decidual microenvironment from abortion-prone and normal pregnancies on murine natural killer cells

Seyed Mohammad Javad Mirarefin[1], Seyed Mohammad Moazzeni[1]

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University

Background: Recurrent miscarriage and implantation failure are the most common complications of pregnancy. Immunological dysregulation is one of the main incidence factors of recurrent spontaneous abortion (RSA). Uterine NK cells are the major immune cells at the maternal-fetal interface and play an important role in the establishment and maintenance of pregnancy. Changes in the NK cell frequency and phenotype were reported in association with RSA by other investigators, but the main regulatory factors for these NK cell variations are not cleared yet. This study was conducted to clarify the effects of decidual microenvironment factors of abortion-prone and normal pregnant mice on the phenotype of NK cells.

Methods: Female CBA/J mice were mated with male DBA/2(abortion-prone) or BALB/C (normal pregnancy) mice. After confirming the onset of gestation, the gravid CBA/J mice were sacrificed on day 13.5 of pregnancy, and the decidual tissue was isolated. Splenic NK cells were purified from the CBA/J mice by magnetic bead separation method, and co-cultured with decidual-derived single cells. Finally, the NK cells were examined for their phenotype and expression of inhibitory and activating receptors by flow cytometry.

Results: The phenotype of NK cells following co-culture with decidua of the abortion-prone mouse model was different from NK cells cultured with normal pregnant decidua. The decidual microenvironment of abortion-prone mice caused decreased expression of NKP46 and KLRG1 and increased expression of NKG2D on NK cells compared to normal pregnant decidua. While the expression Ly49G2 was the same on the NK cells treated by both types of the decidual microenvironment.

Conclusion: The uterine microenvironment could be considered the chief regulator of pregnancy outcome which can affect the phenotype and functions of regional immune cells such as NK cells as the main population of decidual leucocytes which plays important roles in the establishment and maintenance of pregnancy.

Keywords: NK cell, uterine microenvironment, recurrent spontaneous abortion

Effect of 1, 25 $(OH)_2$ D3 on Proliferation, cell cycle, and Apoptosis in endometriotic patients

Ali Akbar Delbandi[1], Nesa Rashidi[1], Maryam Sadri[1], Soheila Arefi[2]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran, Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
2. Genetics and In Vitro Assisted Reproductive (GIVAR) Center, Erfan Hospital, Tehran, Iran

Background: Endometriosis, which is characterized by the growth of endometrial tissue outside the uterus, could result from increased proliferation or decreased apoptosis. According to the antiproliferative and proapoptotic effects of 1,25(OH)2D3 on different cancer cells, in this study, we evaluate the 1,25(OH)2D3 effects on proliferation, cell cycle, and apoptosis of endometriotic stromal cells from endometriotic patients compared with healthy groups.

Methods: ESCs (Endometrial Stromal Cells) isolated from 10 women with endometriosis and 10 healthy controls were treated with 1, 25$(OH)_2$ D3. The proliferation of control endometrial stromal cells (CESCs), eutopic endometrial stromal cells (EuESCs), and ectopic endometrial stromal cells (EESCs) were analyzed using methyl thiazolyl tetrazolium assay. Propidium iodide staining and flow cytometry were used to determine the cell cycle distribution in ESCs. Annexin V/propidium iodide double staining was used to evaluate apoptosis in ESCs.

Results: In the presence of estrogen, 1, 25$(OH)_2$ D3 treatment inhibited the proliferation of ESCs from all three origins ($P < 0.01$ for CESCs and EuESCs and $p<0.001$ for EESCs). The percentage of S phase cells in EESCs was higher than in EuESCs and CESCs ($p<0.01$). The percentage of G1 phase cells in EESCs was lower than that of EuESCs and CESCs ($p<0.01$) and the percentage of G1 phase cells in EuESCs was lower than that of CESCs ($p<0.01$). Moreover, 1, 25$(OH)_2$ D3 inhibited the cell cycle regardless of cell type ($P < 0.01$ in EESC and EuESCs and $p<0.05$ in CESCs). In addition, 1, 25$(OH)_2$ D3 induces apoptosis in EuESCs and EESCs ($p<0.01$).

Conclusion: Although 1,25(OH)2D3 increased apoptotic and necrotic cells and decreased live cells in the EuESCs and EESCs, it did not affect apoptosis in CESC and only increased necrotic cells. These findings indicate that 1,25(OH)2D3 potentially has a growth-inhibiting and pro-apoptotic effect on ESCs from endometriotic patients.

Keywords: ESCs, Proliferation, Cell Cycle, Apoptosis

Effect of active vitamin D3 on estrogen synthesis and signaling pathway in endometriotic patients

Ali Akbar Delbandi[1], Nesa Rashidi[1], Maryam Sadri[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Endometriosis is a gynecological disorder characterized by the growth of endometrial tissue (glands and stroma) outside the uterus and shows estrogen dependency and progesterone resistance. It has been demonstrated that hormonal alterations in endometriosis are involved in the pathogenesis of the disease. In this study, due to the anti-estrogenic effects of vitamin D3 on cancer cells and according to the destructive effects of estrogen in endometriosis, we investigated the effect of vitamin D3 on estrogen signaling and synthesis in endometriosis.

Methods: Control endometrial stromal cells (CESCs) from 10 healthy individuals, and eutopic endometrial stromal cells (EuESCs) and ectopic endometrial stromal cells (EESCs) isolated from 10 women with endometriosis were evaluated for the expression of estrogen receptor α(ER-α), estrogen receptor β(ER-β), GPR30, Aromatase, PGES (prostaglandin E synthase), as well as the steroid receptor activator (SRC) family member, SRC1. Using Real-Time PCR and Western Blotting techniques, we investigated gene and protein expression of mentioned molecules in the presence and absence of 1,25(OH)2D3.

Results: Real-Time PCR analysis demonstrated that gene expression of ER-βand Aromatase in EESCs was higher than EuESCs ($p<0.05$ and $p<0.001$, respectively). Also, gene expression of SRC1, Aromatase, and PGES in EESCs was higher than CESCs ($p<0.0001$, $p<0.001$, and $p<0.01$, respectively) and gene expression of SRC1 in EuESCs was higher than CESCs ($p<0.0001$). Western blotting analysis demonstrated that protein expression of ER-β, SRC1, and GPR30 in EESCs was higher than EuESCs ($p<0.01$). Also, protein expression of ER-β, GPR30, and Aromatase in EESCs was higher than CESCs ($p<0.05$ for ER-β and $p<0.01$ for GPR30 and Aromatase), and protein expression of SRC1 and Aromatase in EuESCs was higher than CESCs ($p<0.05$ and $p<0.01$, respectively). Also, 1, 25(OH)2D3 reduced the gene expression of ER-βin EESCs and protein expression of ER-βin CESCs, EuESCs, and EESCs ($p<0.01$). 1,25(OH)2D3 significantly reduced the gene expression of GPR30 and gene and protein expression of Aromatase in the cells from all origins and protein expression of GPR30 in EuESCs and EESCs.

Conclusion: 1,25(OH)2D3 decreased the gene and protein expression of molecules that are involved in the synthesis and signaling of estrogens such as ER-β, GPR30, Aromatase, and PGES. The findings of this study showed that vitamin D3 has anti-estrogenic effects on ESCs from endometriotic patients. This study sheds new light on a possible approach to endometriosis treatment for both scientists and clinicians.

Keywords: 1, 25(OH)2D3, estrogen, endometriosis

EP4 promotes immune inflammation through Th1 cell differentiation in abortion-prone mice

Fatemeh Tatari[1]

1. *Department of Immunology, Lorestan University of Medical Sciences, Khorramabad, Iran.*

Background: Recurrent spontaneous abortion is defined as three or more consecutive spontaneous abortions before the 20th week of pregnancy, which affects 2 to 5% of couples. One of the main causes of miscarriage in the first trimester of pregnancy is impaired maternal immune system responses. Changes in the expression of prostaglandin receptors during pregnancy and childbirth are important to maintain the conditions of the uterine environment for pregnancy or childbirth. Recent studies have shown that EP2 and EP4 signaling increases Th1 differentiation. Although in most cases, cytokines are involved in Th cell development, T cell differentiation in vivo may be influenced or be strengthened by non-cytokine material in the local inflammatory microenvironment. Some studies have examined how the local microenvironment of the uterus affects Th cell differentiation and the mechanisms that regulate these cells during pregnancy. The role of EP4 in promoting inflammation is known and in this study, the relationship between the expression of this receptor and abortion has been investigated.

Methods: At the end of each phase of pregnancy, placental tissue was isolated in abortion-prone and control mice. After mRNA extraction and cDNA synthesis, the expression levels of EP4, T-bet, and GATA-3 genes in the early gestation (day 9.5), the mid-gestation (day 13.5), and the late-gestation (day 17.5) were measured by qRT-PCR.

Results: The results showed a significant increase in the relative expression of the EP4 gene in mice prone to abortion in the late-gestation compared to mice with normal pregnancy (P=0.045). Also, the relative expression of the T-bet gene had an elevation in mice prone to abortion in the late-gestation compared to mice with normal pregnancy (P=0.026).

Conclusion: These results suggest that EP4 may be involved in abortion by inducing Th1 cells through increasing T-bet expression.

Keywords: abortion-prone mice, EP4, T-bet

Evaluation of Anti paternal cytotoxic antibodies in patients with recurrent spontaneous abortion (RSA) in comparison with normal mothers

Ahad Zare[1,2]

1. *Sarem Fertility and Infertility Research Center (SAFIR), Sarem Women's Hospital, Iran University of Medical Science s (IUMS), Tehran, Iran. Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran.*
2. *Department of Immunology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.*

Background: The immunological relationship between mother and fetus is a two-way communication that is determined by the presence of fetal antigens and identifying the mother's immune system response to these antigens. Some studies have shown that most women with a history of recurrent miscarriage due to alloimmune disease have human leukocyte antigens (HLA) in common with paternal HLA, and this similarity may prevent the formation of an appropriate immune response during pregnancy. These patient doesn't develop blocking antibodies such as anti-paternal cytotoxic antibodies (APCA). The lack of formation of these antibodies is considered the main cause of abortion. In other words, during a normal pregnancy, the maternal immune system often detects paternal antigens as foreign agents and produces various alloantibodies such as APCA, Ab-2, and MLR-Bf that cover the fetal surface and protect it from the maternal immune response. Absence or decreased expression of these alloantibodies during pregnancy may lead to RSA. The aim of this study was the evaluation of Anti paternal cytotoxic antibodies in RSA patients in comparison with normal mothers.

Methods: This descriptive cross-sectional study was performed on 77 volunteer subjects with RSA history referred to Sarem women's hospital (Tehran, Iran) and 37 healthy women without a history of abortion and having at least one live child. The peripheral blood mononuclear cells (PBMCs) were isolated from their husband and were injected into the patients, two or three times. Two weeks after the last immunization, the patients' sera were tested for anti-paternal cytotoxic antibodies by leukocyte cross-match test (WBC cross-match).

Results: WBC cross-match test was 5.2±1.8 in RSA and 32±9.5 in the control group. These results show that the rate of APCA in patients with recurrent miscarriages is significantly lower than in healthy women.

Conclusion: According to our findings in this study, it is important to evaluate the blocking antibodies such as Anti paternal cytotoxic antibodies in recurrent spontaneous abortion patients. Also, Lymphocyte therapy which can enhance the production of APCAs in patients with RSA is recommended for these patients because our previous study showed, this treatment can increase the possibility of positive cross-match tests in RSA patients.

Keywords: Recurrent spontaneous abortion; APCA; WBC cross match

Evaluation of CD3+ T-cells Percentage and Function and its Relationship with Serum Vitamin D Levels in Women with Recurrent Spontaneous Abortion and Recurrent Implantation Failure

Abdolah Mousavi Salehi[1], Mehri Ghafourian[1], Afshin Amari, Mahvash Zargar[2]

1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Fertility, Infertility and Perinatology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Background: Women with recurrent spontaneous abortion (RSA) and repeated implantation failure (RIF) may have immune abnormalities. Vitamin D is known to play a role in the function of the immune system. This study aimed to evaluate the percentage and function of CD3+ T-cells and their relationship with the serum vitamin D level or 1,25-dihydroxy vitamin D3 (an active form of the vitamin) in women with RSA and RIF.

Methods: PB was obtained from the patient and healthy control groups in this case-control study. The percentage of CD3+ T-cells and activated CD3+ CD69+ T-cells was investigated using flow cytometry. Serum levels of Interferon-γ (IFN-γ) and vitamin D were also measured using the Enzyme-linked immune assay (ELISA) technique.

Results: The mean percentage of CD3+ T-cells in women with RSA increased significantly compared to the healthy control group ($P<0.04$). However, no significant difference was observed in RIF women compared to the control group. There was no significant difference in the percentage of activated CD3+CD69+ T-cells between the patient and healthy control groups. Serum IFN-γ levels in women with RSA showed a significant increase compared to the control group ($p<0.031$); however, no significant difference was observed between women with RIF and the control group. Serum levels of vitamin D showed a significant decrease in both RSA ($p<0.01$) and RIF ($p<0.04$) groups relative to the control.

Conclusion: As a result, we found an increase in T-cell percentage and inflammatory function. Vitamin D deficiency can lead to immune system dysfunction and pregnancy complications.

Keywords: T- cells, recurrent spontaneous abortion, recurrent implantation failure, interferon- γ, vitamin D

Evaluation of complement system function and C3 and C4 levels in recurrent spontaneous abortion patients

Ahad Zare[1, 2]

1. *Sarem Fertility and Infertility Research Center (SAFIR), Sarem Women's Hospital, Iran University of Medical Science s (IUMS), Tehran, Iran. Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran.*
2. *Department of Immunology, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.*

Background: Recurrent miscarriage is the most common pregnancy problem and is often defined as two or more recurrent miscarriages before the twentieth week of pregnancy. The complement system with the mechanism of lysis of bacteria and extracellular pathogens focuses on the elimination of these foreign agents and can be effective in regulating normal pregnancy and preserving or rejecting the semi-allogeneic fetus. The aim of this study is evaluation of the function of the complement system and the levels of C3 and C4 in patients with recurrent miscarriages.

Methods: In this case-control study, two groups were referred to Sarem Hospital in Tehran in the period from 2020 to 2021 with 77 female patients with a history of at least three recurrent spontaneous abortions as a group and 37 healthy women without a history of abortion and at least one live child were included in the study as a control group. Patients were evaluated for karyotype, anti-phospholipid antibody, anti-cardiolipin, ANA, anti-thyroid antibody, recurrent abortion-related infections, anatomical abnormalities, and hormonal disorders and included in this study. To measure the activity of the complement system, the CH50 test was performed using the SRID method, and the levels of C3 and C4 were detected by the nephelometry method. The measured values were analyzed by SPSS software version 22.

Results: The present study showed that the levels of complement proteins, C3 and C4, were significantly reduced in women with recurrent miscarriages compared to the control group. Also, the comparison of complement activity in these two groups shows less serum complement activity in patients with a history of recurrent miscarriage.

Conclusion: Considering the possibility of complement proteins involved in recurrent spontaneous abortion, examining the level of major complement proteins such as C3 and C4 and measuring the serum complement activity of these patients can be useful in diagnosing immunological abortions.

Keywords: Recurrent Miscarriage; Complement System; Pregnancy Failure; C3 and C4 Proteins

Evaluation of platelet-rich plasma (PRP) therapy in a patient with a history of IVF failure

Sahar Salimi[1], Abu Taleb Saremi[2], Nasim Atef Yekta[3], Maryam Shami[1], Narges Roomandeh[3], Paniz Baghshahi[3], Ahad Zare[4]

1. Sarem Gynecology, Obstetrics, and infertility research center, Sarem Women's Hospital, Iran University of Medical Sciences, Tehran, Iran
2. Sarem Gynecology, Obstetrics, and infertility research center, Sarem Women's Hospital, Iran University of Medical Sciences, Tehran, Iran.Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran
3. Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran
4. Department of Microbiology and Immunology, Faculty of medicine. Tehran medical sciences, Islamic Azad University, Tehran, Iran

Background: One of the most recent applications of platelet-rich plasma (PRP) is improving pregnancy in IVF through increasing endometrial growth. It has been observed that autologous PRP injection causes endometrial growth and thus improves pregnancy. Also, it has been reported that PRP has an important role in improving pregnancy in patients with repeated implantation failure (RIF). PRP has immunomodulatory roles with regulatory T cell induction and activated NK cell suppression.

Materials and methods: In this study, the effectiveness of this treatment has been evaluated by examining patients with a history of RIF. This cross-sectional descriptive study was conducted on 2,260 patients with a history of RIF referred to Sarem Hospital for IVF treatment between 2020-2022. 341 patients volunteered for treatment and 1919 patients were considered as the control group. Platelet separation was performed in two-step centrifugation.

Results: Examining the IVF results of volunteer patients with PRP treatment showed that 46 patients of 341 volunteer patients (13.48%) had a successful pregnancy after the 20th week. Evaluating the results of IVF in 1919 other patients were used as a control group, which also showed that 215 patients (11.2%) passed the 20th week of pregnancy with positive ultrasound and HCG test results.

Conclusion: Comparing the results of patients with a history of RIF with the control group shows that this type of treatment does not make a significant difference in the pregnancy outcome.

Keywords: PRP, IVF

Evaluation of serum Peroxiredoxins auto antibodies in recurrent spontaneous abortion patients

Sahar Salimi[1], Narges Roomandeh[1], Abu Taleb Daremi[2], Mohammad Mehdi Adibzadeh Sereshgi[1], Ahad Zare[3]

1. Sarem Gynecology, Obstetrics, and infertility research center, Sarem Women's Hospital, Iran University of Medical Sciences, Tehran, Iran
3. Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran
3. Department of Microbiology and Immunology, Faculty of medicine. Tehran medical sciences, Islamic Azad University, Tehran, Iran

Background: The most common complication in pregnant individuals is Recurrent spontaneous abortion (RSA) in which autoantibodies have been also observed. Autoantibody against Peroxiredoxin 3 (Prx3) and Peroxiredoxin 4 (Prx4) as placental antigens were recognized in RSA patients. Autoantibodies could have destructive effects on Pr3 and Pr4 antioxidant activity. The present scientific research aimed to evaluate the quantitative assessment of autoantibodies in the serum of RSA patients.

Methods: 100 volunteers with a history of at least three RSA and 32 women with at least two successful pregnancies without any abortion as a control group were included. The serum level of Anti-Prx3 and anti-Prx4 were measured with ELISA. Results were analyzed with an independent sample t-test.

Results: The data indicated that the level of anti-Prx4 is higher in RSA patients as compared to normal controls (p=0.004). As well, there was no statistical difference in the level of anti-Prx3 between RSA and healthy women (p=0.51).

Conclusion: The results demonstrated that anti-Prx4 auto antibody as a novel immunologic parameter increases in RSA patients. This finding could be considered a diagnostic immunological marker in RSA patients.

Keywords: anti-Peroxiredoxin3, anti-Peroxiredoxin4, Recurrent spontaneous abortion

Evaluation of Th17 cell frequency and expression of microRNAs and Th17-related factors in patients with premature ovarian failure (POF)

Narjes Nouri[1], Leili Aghebati-Maleki[2], Shahla Danaii[3], Mohammad Sadegh Soltani-Zangbar[1], Amin Kamrani[1], Mehdi Yousefi[4]

1. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
3. Gynecology Department, Eastern Azerbaijan ACECR ART center, Eastern Azerbaijan branch of ACECR, Tabriz, Iran.
4. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Folliculogenesis is a normal process during which follicles grow regularly, and finally, ovulation occurs. If folliculogenesis changed, it can lead to POF, which is one of the common problems of women. It has been discovered that miR-326 stimulates Th17 differentiation by targeting Ets-1, a negative regulator of Th17 differentiation. Th17 cells lead to an increase in the immune system's response and the secretion levels of IL-17, which plays a role in inflammation and autoimmune diseases. Inflammation caused by Th17 and loss of balance between Th17/Treg can lead to POF. This study aimed to investigate the effect of inflammation in causing premature ovarian failure.

Methods: In this study, 30 patients with POF and 30 healthy individuals were sampled. After isolating peripheral blood mononuclear cells (PBMC), the number of Th17 cells, expression levels of miR-326 and Th17 cells related transcription factor and cytokines, and also the secretory levels of these cytokines investigated respectively by Flow cytometry, Real-time PCR and ELISA.

Results: Compared to the control group, our results showed a significant increase in the number of Th17 cells along with an increase in the transcription factor RORγt in patients with POF. The secretion levels of inflammatory cytokines (IL-17, IL-21, and IL-23) in PBMC of patients with POF significantly increased compared to the control group. The results of our study also showed an increase in expression levels of miR-326 in women with POF.

Conclusions: The increase in pro-inflammatory parameters in women with POF compared to the control group indicate the undeniable role of the immune system in the process of pregnancy disorders. Therefore, immunological factors can be used as potential biomarkers in the prognosis of women with a high risk of POF soon.

Keywords: Premature ovarian failure, RORγt, T helper 17, microRNA

Evaluation of Vα7.2-Jα33, TNF-α, IL-17A, IFN-γ mRNA expression in eutopic and ectopic tissue of endometriosis patients

Maryam Moghaddam[1], Ali Shams[1], Fateme Zare[2], Reyhane Sandoghsaz[3]

1. Department of Immunology, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran,
2. Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Background: Endometriosis is a chronic estrogen-related inflammatory disorder that is known by replacing and proliferating endometrial and stromal gland cells in a place outside the uterus, often in the pelvis and ovarian tissue. The high presence of innate and acquired immune cells in the peritoneal fluid of women with endometriosis confirms the involvement of the immune system in the pathogenesis of the disease. Vα7.2-Jα33 Mucosal-Associated Invariant T (MAIT) cells play an undeniable impact on mucosal immunity by the production of IL-17, IFN-γ, and TNF-α. The function of the cells in the pathogenesis of endometriosis s less investigated. Objective: The study aims to investigate of infiltration of MAIT cells by using the determination of levels of Vα7.2-Jα33 genes expression in eutopic and ectopic tissue of endometriosis lesions. Essential inflammatory cytokines including TNF-α, IL-17A, and IFN-γ mRNA expression also is measured in the tissues. The obtained results compared with healthy age-matched control.

Methods: In the case-control study, 20 patients with endometriosis and 20 healthy non-endometriosis women of reproductive age were recruited. The tested samples include 20 eutopic tissues and 20 ectopic tissues of women with endometriosis and 20 uterine endometrial tissues of women in the control group. RNA was extracted from the tissues and cDNA was synthesized according to standard protocols. Expressions of the desired genes were analyzed by quantitative reverse transcriptase (q-RT)-PCR. β2-microglobulin gene was used as an internal control. Independent Student's t-test was used to compare gene expression in groups.

Results: According to the study's results, expression of Vα7.2-Jα33 genes did not show a substantial elevation in the uterine and eutopic endometrial tissues compared to internal gene control as well as in ectopic tissues. Statistical analysis showed significant elevation ($p<0.05$) in IFN-γ and IL-17A gene expression in ectopic tissue compared to eutopic and uterine endometrium tissue. Correlation analysis approved a positive relationship between Vα7.2-Jα33 expression genes and IFN-γ levels in ectopic tissues. However, levels of TNF-α and IL-17A did not show a correlation with Vα7.2-Jα33 expression genes in the ectopic tissues.

Conclusion: Considering the low expression specific gene of MAIT cells in ectopic tissue, it can be concluded that these cells present in the endometriotic environment to a small extent, and there is a possibility of their role in the progression of endometriosis by secreting IFN-γ.

Keywords: Endometriosis, MAIT, IFN-γ, TNF-α, Vα-7.2-Jα33, IL-17

Frequency changes in decidual myeloid cells of abortion-prone mice following adipose-derived mesenchymal stem cells therapy

Setareh Karimi[1], Seyed Mohammad Moazzeni[1]

1. *Tarbiat Modarress University, Tehran, Iran.*

Background: Failure of immunological tolerance to the semi-allogeneic fetus is one of the main causes of recurrent spontaneous abortions (RSA). Past studies have shown that imbalance in myeloid cells' frequency at the feto-maternal interface plays a remarkable role in abortions. An abnormal abundance of infiltrating myeloid cells has been proven in implantation sites of abortion-prone mice during early pregnancy causing increased pro-inflammatory mediators and impaired spiral artery remodeling which leads to disorder in implantation, and abnormal trophoblast invasion. Our previous studies showed that adipose-derived mesenchymal stem cell (AD-MSC) therapy reduces the abortion rate in CBA/J × DBA/2 matings through immunomodulatory properties.

Methods: This study was designed to evaluate the effects of AD-MSCs on myeloid recruitment to the implantation site. MSCs isolated from CBA /J adipose tissues were intraperitoneally injected at day 4.5 of gestation to the abortion-prone group. Similarly, PBS was administered to the control group. The placental tissues were harvested from CBA/J females on day 13.5 of gestation after evaluation of the resorption rate. The frequency of myeloid cells was evaluated in paraffin-embedded tissue sections by Immunohistochemistry methods.

Results: Our results indicate the percentage of miscarriage decreased in the treatment group compared to controls. We also found that the frequency of CD11b positive cells in the treatment group was significantly reduced compared to the controls.

Conclusion: Our results suggest that the AD-MSC therapeutic approach results in regulating the function and frequency of decidual myeloid cells and improve the tolerogenic microenvironment at the feto-maternal interface for maintaining the semiallogeneic fetus.

Keywords: Mesenchymal Stem Cells, Recurrent Spontaneous Abortion, Cell Therapy, CD11b+ Myeloid Cells

Investigating the frequency of CD4+ and CD8+ memory lymphocyte populations (central, effector, stem cell) in the peripheral blood of women with successful IVF, failed IVF, and women with normal pregnancies

Maryam Sadat Hasheminasab[1], Behrouz Gharesi-Fard[2], Nader Bagheri[2]

1. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
2. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

Background: Infertility is the failure to achieve a clinical pregnancy after 12 months or more of regular and unprotected intercourse. In vitro fertilization (IVF) is one of the most invasive and costly infertility treatments. During normal pregnancy, human decidua contains a large number of immune cells, the balance of which can lead to the tolerance of fetal antigens and therefor a successful pregnancy. Memory T cells are important immunological cells participating in pregnancy. This study aimed to evaluate the frequencies of CD4+ and CD8+ memory T cells in the peripheral blood of infertile women with successful or unsuccessful IVF and fertile women with normal pregnancies.

Methods: A total of 45 people (17 infertile women with a history of IVF failure, 13 infertile women with a history of successful IVF, and 15 healthy pregnant women) were included in this study. The abundance of CD4+ and CD8+ memory T cells (central, effector, stem cell, and TEMRA) in peripheral blood mononuclear cells (PBMC) was measured using flow cytometry.

Results: The results showed that the frequency of CD4+ and CD8+ central memory T cells in women with successful IVF was significantly higher than in women with a history of IVF failure or women with normal pregnancies ($p=0.001$ and $p=0.01$, respectively). There was also no significant relationship between the frequency of effector memory cells, memory stem cell, and TEMRA among the three groups.

Conclusion: Increasing the level of central memory T cells might improve IVF outcomes.

Keywords: Memory cells, Effector memory T cell, Central memory T cell, IVF

Investigation of the immunomodulatory effects of nanocurcumin on peripheral blood mononuclear cells (PBMCs) of patients with preeclampsia, an in-vitro study

Samaneh Abdolmohammadi-Vahid[1], Mehdi Yousefi[2], Leila Azhdari-Habashi[3], Ali Aghebati-Maleki[4], Leili Aghebati-Maleki[3]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
3. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4. Stem Cell Research Center, Tabriz University of Medical Science, Tabriz, Iran

Background: Preeclampsia, characterized by hypertension and proteinuria, is among the most common complications of human reproduction, which may lead to maternal and neonatal morbidity. Dysregulation of the maternal immune system such as disturbed immunoregulation and elevated inflammation, has been proposed to play a role in the pathogenesis of preeclampsia. Nanocurcumin, a nano-scaled formulation of curcumin, has been confirmed to have anti-inflammatory and anti-oxidative effects. We aimed to investigate the effect of nanocurcumin on T helper (Th17)/regulatory T cells (Treg) balance among PBMCs of patients with preeclampsia.

Methods: Thirty patients with preeclampsia and 30 healthy pregnant women were enrolled. 12 mL of peripheral blood was obtained from all the participants. The frequency of Treg and Th17 cells was evaluated by flowcytometry. PBMCs were exposed to different concentrations of nanocurcumin, to determine the cytotoxicity effect of nanocurcumin by MTT assay. Treg and Th17-associated transcription factors gene expression and cytokine secretion were evaluated by real-time PCR and ELISA, with and without nanocurcumin treatment.

Results: Patients with preeclampsia had a higher frequency of Th17 ($p=0.0005$) and a lower frequency of Treg cells ($p=0.01$), in comparison with the control group. Upon incubation of PBMCs of preeclampsia patients with nanocurcumin, Th17-associated transcription factors, RORγt ($p=0.02$), and STAT3 ($p=0.01$) were significantly decreased, when compared to untreated PBMCs of these patients. Moreover, secretion of IL-6 ($p= 0.02$), IL-17 ($p= 0.04$), IL-21 ($p= 0.02$), and IL-23 ($p= 0.03$) were also downregulated. In contrast, nanocurcumin significantly increased the gene expression of FoxP3 ($p= 0.03$) and secretion level of IL-10 ($p= 0.04$) and TGF-β ($p= 0.04$) in PBMCs of patients.

Conclusion: Regarding the immunomodulatory effect of nanocurcumin, this substance may be a helpful therapeutic choice in the modulation of aberrant immune responses observed in preeclampsia and it may reduce the unfavorable outcomes of pregnancy in these patients.

Keywords: Preeclampsia, Nanocurcumin, Regulatory T cells, T helper 17

Investigation of the frequencies of TCD4+ memory cell subsets in pregnant women complicated with lupus

Leila Bordbar[1], Andisheh Mosaffa Jahromi[1], Niloofar Namazi[2], Bahia Namavar Jahromi[3], Moslem Ahmadi[1], Behrouz Gharesi-Fard[1]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Gynecology and Obstetrics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Background: Systemic lupus erythematosus (SLE) is a chronic multi-system autoimmune disease. Pregnant women with SLE are at higher risk for pregnancy complications. Previously published studies have highlighted the role of CD4+ memory T cells in the success or failure of pregnancy. Although no study has been conducted on the frequency of memory T cells in pregnant women with lupus yet. Therefore, we aimed to investigate the frequency of CD4+ memory T cell subsets including CD4+ TEM, TCM, TFHM, TSCM, and TEMRA cells in the peripheral blood of women who simultaneously engaged with lupus and pregnancy.

Methods: Fifteen healthy women with normal pregnancies and twenty-eight women who suffered from lupus with mild or severe pregnancy complications participated in this study. The CD4+ memory T cell subsets were assessed by four colors flow cytometry method.

Results: Results indicated that the frequency of CD4+ CD45RO- CCR7- TEMRA cells was significantly higher in pregnant women with lupus compared to healthy control ($p=0.01$), also a significantly higher frequency of CD4+ CD45RO- CCR7- TEMRA cells was seen in women with lupus with mild pregnancy complications compared to healthy control ($p=0.02$). Moreover, the frequency of CD4+ CD45RO+ CCR7+ TCM cells was significantly higher in the group of women with lupus and abortion compared to the group with mild pregnancy complications ($p=0.03$).

Conclusion: In conclusion, the results of the present study showed that the frequency of TCD4+ memory subsets might be important in the outcome of pregnancy in women with lupus.

Keywords: Memory T cell subsets, lupus, pregnancy complications

Mesenchymal stem cells therapy changes the decidual microenvironment to modulate the dendritic cells' properties for the benefit of pregnancy maintenance

Maryam Eskandarian[1], Seyed Mohammad Moazzeni[1]

1. Tarbiat Modares University, Tehran, Iran.

Background: Recurrent spontaneous abortion (RSA) is one of the well-known complications of pregnancy. The malfunctions of immune cells such as dendritic cells (DCs) are proposed as the creative mechanism in most RSA cases. Mesenchymal stem cells (MSCs) are immunomodulatory cells with the potency of modulating the immune cells' properties and function. This study was done to clarify the effects of MSCs therapy on the decidual microenvironment and accordingly on DCs as one of the most important cells in the establishment and maintenance of pregnancy.

Methods: Adipose-derived mesenchymal stem cells were administered to abortion-prone pregnant mice (CBA/J ×DBA/2). In the control group, the PBS was injected. The decidual cells from MSCs-treated and non-treated pregnant mice were co-cultured with bone marrow differentiated immature DCs in the presence of paternal antigen. The immunophenotype, antigen uptake properties, and antigen-specific T cell stimulation potency of DCs were measured through the flowcytometry analysis. The expression levels of IFN-γ, IL-10, TGF-β, and TNF-α mRNA in dendritic cells were also determined by real –time PCR.

Results: Our results indicated that decidual cells from the MSC-treated group had an inhibitory effect on DC maturation and function. A significant reduction in the expression of co-stimulatory molecules (MHC-II, CD86, and CD40) and induction of T cells proliferative response by DCs were observed in DCs co-cultured with the decidual cells of MSCs treated group compared with non-treated group. However, the Ag uptake capability of DCs has remained unchanged. Furthermore, decidua from MSC- treated mice noticeably decreased the ability of DCs for inducing IFN-γ and TNF-α production whereas the secretion of TGF-β and IL-10 remarkably increased compared with control mice.

Conclusion: Collectively, our results showed the obvious potency of MSCs therapy to modulate the decidual microenvironment for the benefit of regulating the dendritic cells' phenotype and functions to establish an immune protective milieu for the fetus and maintenance of pregnancy.

Keywords: Dendritic cell, abortion, pregnancy, MSCs therapy, decidual microenvironment

Patients with idiopathic Recurrent Pregnancy Loss and have abnormalities in blood NK, NKT, and T cells

Vida Homayouni[1], Pezhman Beshkar[2], Ali Ajami[3], Parinaz tavakoli Dinani[3], Elaheh Vaez Moghadam[3], Asiyeh Masaeli[3]

1. Immunodeficiency Diseases Research Center, Isfahan, University of Medical Sciences, Isfahan, Iran. Department of Immunology
2. Molecular & Cellular research center, basic health science institute, Shahrekord University of Medical Science, Shahrekord, Iran
3. Nobel Laboratory, Isfahan, Iran.

Background: Recurrent Pregnancy Loss, defined as two or more miscarriages affects up to 1.9% of couples. Almost, immune dysregulation is a reason. Peripheral Blood Markers in particular Natural killer cells and T cells can be a suitable prognostic marker for the early detection of unexpected complications during abortion. Understanding changes in the immune profile of patients with idiopathic recurrent pregnancy loss may lead to new possibilities in therapeutic intervention or immune medication.

Methods: Blood samples from 25 iRPL patients were obtained during visits to the clinician's office. Healthy blood donors were gathered as controls. The samples were stained with fluorochrome-labeled monoclonal antibodies CD3, CD56, CD16, CD4, and CD8 and incubated for 30 minutes at room temperature in the dark. Cells were lysed, washed, and read by flow cytometry.

Results: We compared the results between iRPL and controls. A p-value of <0.050 showed a significant study and reflected the imbalance of the immune system in iRPL patients.

Conclusion: iRPL patients showed an abnormally high cytotoxic NK cell dim and NKT. Indeed, this imbalance is considered a long-term immune response against a persisting antigen and speculated that this cytotoxic immune response is directed towards the fetus. Therefore, immune cell testing is required to permit accurate identification of those women who may benefit from immunomodulation.

Keywords: iRPL, NKT, NKdim, Miscarriage

Reduced frequency of CD4+FOXP3+CD39+CD73+regulatory T cells and gene expression of adenosine 2A receptor in peripheral blood mononuclear cells of unexplained infertile females

Maryam Rezanejadamirdehi[1], Mousa Mohammad Nia-Afrouzi[2], Abdolreza Darai[1], Sedigheh Esmaeilzadeh[3], Mehdi Shahbazi[4]

1. Immuno regulation Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
2. Department of Immunology, School of Medicine, Babol University of Medical Sciences, Babol, Iran
3. Infertility and Health Reproductive Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
4. Immuno regulation Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

Background: Unexplained infertility (UI), infertility without a known cause, is observed among infertile couples in the word. 10-17% of infertile women are UI. Regarding the play of the immune system and regulatory T cells (Treg) in the reproductive system, these cells have shown a necessary role during on initiation of implantation and normal pregnancy. The most regulatory feature of these cells is the demonstration of TCD4+FOXP3+CD39+CD73+ a regulatory cells and expression of adenosine 2a receptor (A2AR). These cells and receptors could cause maternal immune tolerance against the semi-allogenic fetus.

Methods: Fifty-five samples, 30 UI females and 25 fertile females were selected and their demographic data was recorded. 5 ml peripheral blood samples were collected from the two mentioned groups. PBMCs were purified by using the Ficol method. Then the frequency of CD4+FOXP3+CD39+CD73+ lymphocytes was evaluated by adding fluorochrome-conjugated antibodies against CD4, FOXP3, CD39, and CD73 molecules. Expression levels of gene adenosine 2a receptor in peripheral blood mononuclear cell involved with RT-PCR.

Results: Our results showed that the frequency of CD4+FOXP3+ T lymphocytes decreased in females with UI in comparison with fertile females, it was significant statistically (P=0.0001). Also the percentage of CD4+FOXP3+CD73+ T lymphocytes was higher in fertile females in comparison with UI females, it was significant ($p=0.005$). the percentage of CD4+FOXP3+CD39+ T lymphocytes decreased in females with UI in comparison with fertile females, it was shown ($p=0.0001$). the percentage of CD4+FOXP3+CD39+CD73+ T lymphocytes decreased in females with UI in comparison to the control group, it was significant statistically ($p=0.001$). The finding demonstrated that despite the low percentage of CD4+ T lymphocytes in females with UI in comparison with fertile females, a significant difference wasn't observed($p=0.8$). The level of Adenosine 2a receptor expression in the control group was significantly more than the case group ($p=0.02$).

Conclusion: Our study showed a diminished level of regulatory T cells and Adenosin 2a receptor in UI female compensation healthy control also these regulatory cells seem to be important in implantation although more research needs to about this subject.

Keywords: Regulatory T cell, adenosine 2a receptor, unexplained infertility, Infertility, Immune system

Reduced frequency of uterine group 2 innate lymphoid cells and regulatory T cells in abortion-prone mice

Mohammad Ali-Hassanzadeh[1], Mohammad Dalfardi[2], Ahmad Golkar[2], Mahtab Fasihi[2], Reyhaneh Farrokhi[2], Moslem Ahmadi[3], Behrouz Gharesi-Fard[3]

1. Department of Immunology, School of Medicine, Jiroft University of Medical Sciences, Jiroft, Iran
2. Student Research Committee, Jiroft University of Medical Sciences, Jiroft, Iran
3. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Recurrent spontaneous abortion (RSA) is a complication of pregnancy that affects the physical and mental health of pregnant women and approximately 50% of the mechanism are unclear. Despite hormonal, genetic, and anatomical factors, one of the main causes of RSA is the disruption of the immune response at the feto-maternal interface during the first trimester of pregnancy.

Methods: In this study, we evaluated the frequency of uterine group 2 innate lymphoid cells (uILC2s), their subsets, and uterine regulatory T cells (Tregs) in CBA/J × DBA/2 J as an abortion-prone model compared to normal pregnant (NP) mice using the flow cytometry approach.

Results: In our study, the frequency of uILC2s in the AP group was significantly lower than in the NP group at mid-gestation ($p\leq0.01$). Moreover, the percentages of uterine nILC2s were increased in NP mice at mid-gestation ($p\leq0.05$), while iILC2s significantly increased in AP mice at mid-gestation ($p\leq0.05$). Tregs were reduced in AP mice at both early and mid-gestation stages ($p\leq0.01$).

Conclusion: These data suggest that the changes in uterine ILC2s might be associated with abortion in mice.

Keywords: Pregnancy, Uterus, Innate lymphoid cells, Treg cells, Abortion-prone model

Serum anti-leukemia inhibitory factor antibody and recurrent pregnancy loss in Iranian women

Seyed Mohammad Seifati[1], Hossein Ansariniya[2], Fateme Rahmani[1], Ali Akbar Saboor-Yaraghi[3], Farzaneh Fesahat[1], Hossein Hadinedoushan[1], Fateme Zare[1]

1. Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: This study aimed to determine what is associated with serum anti- leukemia inhibitory factor (LIF) antibody levels in women with recurrent pregnancy loss (RPL) for the first time. Although there are different definitions for RPL, the definition of the health organization is the disorder of the loss of three or more consecutive pregnancies before the 20th week. It has been estimated that between one and four percent of women of reproductive age in Europe and America suffer from this disorder. RPL can be caused by a variety of etiologies, such as uterine factors, thrombophilia, endocrine, physiological, genetic, and immunological factors. The LIF is an interleukin-6 member with a pleiotropic function that plays a critical role in mammalian implantation. A significant reduction in implantation is observed when LIF signaling is inhibited with an antibody or inhibitor.Materials and

Methods: This case-control study was conducted on serum samples of 80 participants. The relationship between the two groups was analyzed using SPSS software.

Results: There was no significant change in the serum levels of anti-LIF antibodies in women with RPL history and the control group.

Conclusion: Several factors are believed to play a role in human infertility, including decreased secretion or mutations of the LIF gene, however, infertility can also occur in women without LIF deficiencies. According to the present study, there was an increased serum anti-LIF antibody level in the case group, but this difference was not statistically significant.

Keywords: recurrent pregnancy loss, leukemia inhibitory factor, antibodies, serum

The assessment of CD39, CD73, and HIF-1α expression and their related miRNAs in Recurrent Pregnancy Loss

Forough Parhizkar[1], Mohammad Sadegh Soltani-Zangbar[2], Forough Chakari-Khiavi[3], Aref Chakari-Khiavi[4], Shahla Danaii[5], Javad Ahmadian-Heris[6], Mehdi Yousefi[1]

1. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
3. Pharmaceutical Chemistry, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
4. Student Research Committee, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
5. Gynecology Department, Eastern Azerbaijan ACECR ART Centre, Eastern Azerbaijan branch of ACECR, Tabriz, Iran.
6. Department of Allergy and Clinical Immunology, Pediatric Hospital, Tabriz University of Medical Sciences, Tabriz, Iran.

Background: There is still a lack of knowledge about the molecular mechanisms involved in recurrent pregnancy loss (RPL). This study aimed to assess the molecules associated with ATP catabolism and hypoxia as well as their related miRNAs in RPL patients.

Methods: The frequency of Treg cells in peripheral blood mononuclear cells (PBMCs) of RPL and healthy pregnant women was determined using Flow cytometry. The expression levels of CD73, CD39, and Hypoxia-inducible factor-alpha (HIF-1α) in PBMCs were quantified using real-time PCR and western blotting. In addition, the expression of miR-30a, miR-18a, and miR-206 was assessed using real-time PCR. After that, the level of TGF-β, IGF-1, and HIF-1α was measured in serum using ELISA. At last, the correlation between evaluated factors was determined.

Results: According to our results, the expression level of CD39 is decreased in PBMCs of RPL cases whereas the level of CD73 and HIF-1α is increased (p=0.0010, p=0.0023, p=0.0006 respectively). Protein analysis confirmed the results of CD39 and CD37 (p=0.0047, 0.0364 respectively). The expression of miR-206 and miRNA-30a is increased (p=0.0038, p=0.0123) and the expression of miRNA-18a is decreased (p=0.0101) in RPL cases. In addition, we indicated a lower level of TGF-β and IGF-1 (p=0.0065, 0.0017 respectively) and a higher level of HIF-1a (p=0.0235) in serum samples of RPL patients compared to healthy cases and their regulation by miRNAs positively or negatively.

Conclusion: We conclude that dysregulation of CD39, CD73, and HIF-1α via changes in the expression of miR-18a, miR-30a, and miR-206 is correlated with decreased frequency of Treg cells in RPL patients. It may be employed for RPL prognosis by more comprehensive future evaluations.

Keywords: Recurrent pregnancy loss; CD73; CD39; HIF-1α; miRNA

The effect of decidual microenvironment from normal and abortion-prone pregnant mice on macrophage phenotype

Nasim Abbasi sibaki[1], Seyyed Mohammad Moazzeni[1]

1. Tarbiat Modares University, Tehran, Iran.

Background: Macrophages are the second dominant population of decidual leukocytes after NK cells. These cells include two subpopulations (M1/M2) that differ from each other in terms of phenotype and function. These cells play a very important role in the success of pregnancy, and any disruption in their sub-populations proportion could lead to pregnancy complications. It is worth mentioning that in recurrent spontaneous abortion (RSA), the dominant population of decidual macrophages is of M1 type, which creates an inflammatory environment, and as a result, pregnancy fails, while in normal pregnancy, the dominant population of decidual macrophages is of M2 type, which is necessary to preserve the fetus. This research is carried out to find that the shift of macrophages towards the M1 and M2 subpopulations is an inherent characteristic of macrophages infiltrating the decidual environment or it takes place under the influence of the decidual microenvironment.

Methods: To investigate the effect of the decidual microenvironment on macrophages, the peritoneal macrophages from female CBA/J mice were co-cultured with decidual cells of normal and abortion-prone pregnant mice. Then the expression of nitric oxide synthetase (iNOS) as an indicator of M1 and arginase-1 (Arg-1) as an indicator of M2 macrophage genes were analyzed by Real-time PCR technique.

Results: Our results showed that abortion- prone decidual cells by creating an inflammatory environment cause the predominant differentiation of peritoneal macrophages to M1 and increase the expression of the iNOS gene, while the effect of decidual microenvironment from normal pregnancy causes the predominant differentiation of macrophages to M2 and increase in Arg-1 gene expression.

Conclusion: According to the obtained results, it seems that the primary factor disrupting the balance of macrophage sub-populations in abortion-prone pregnancies is the decidual microenvironment.

Keywords: macrophage, decidual microenvironment, recurrent spontaneous abortion

Comparison of vitamin D levels in recurrent pregnancy loss patients with and without autoantibodies

Hasan Namdar Ahmadabad[1], Tooba Farazmand[2]

1. Department of Pathobiology and Laboratory Sciences, North Khorasan University of Medical Sciences, Bojnurd, Iran
2. Department of Gynecology, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

Background: The immune dysregulation in recurrent pregnancy loss (RPL) patients may be associated with vitamin D status. The purpose of this study was to compare the vitamin D status in the RPL patients who were seropositive for autoantibodies (autoAbs) including antinuclear antibodies (ANA), anti-phospholipid antibodies (APAs), or anti-thyroid peroxidase (anti-TPO) and RPL patients who were seronegative for them.

Methods: The study groups included: fifty-eight RPL patients who were seropositive for at least one autoAbs (ANA, anti-TPO, or APAs), thirty-four RPL patients who were seronegative for autoAbs, and fifty-eight healthy women who have formerly had at least one successful pregnancy and were seronegative for autoAbs. We measured levels of 25(OH) D through sandwich ELISA in the three groups.

Results: Our results showed that study groups have insufficiency levels of 25(OH) D and serum levels of it in RPL patients with or without autoAbs were significantly lower than in healthy women (P=0.0006). We also found that serum levels of 25(OH) D in RPL patients with autoAbs were significantly lower than in RPL patients without autoAbs (20.51 ± 1.15 ng/ml Vs. 23.69 ± 0.74 ng/ml, P=0.0356). Further analysis indicated that RPL patients who were positive for ANA, APAs, except anti-TPO, had significantly lower than 25(OH)D serum levels compared with RPL patients without autoAbs.

Conclusion: These findings suggest that vitamin D insufficiency is common among RPL patients, especially in RPL patients with positive APAs or ANA. It is possible to hypothesize that decreased 25(OH) D levels may be associated with the induction of autoAb in RPL patients.

Keywords: Vitamin D, Recurrent Pregnancy Loss, Antinuclear antibody, Anti-phospholipid antibody, Anti-thyroid peroxidase

Vitamin D deficiency as a risk factor for endometriosis in Iranian women

Elaheh Abdollahi[1], Ali-Akbar Delbandi[2], Mansour Torab[3], Sepideh Khodaverdi[4], Samaneh Rokhgireh[4], Zahra Moradi[2], Sahel Heidari[2], Tahereh Mohammadi[2]

1. Department of Pharmacology and Toxicology, School of Pharmacy, Iran University of Medical sciences
2. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
4. Endometriosis Research Center, Iran University of Medical Sciences, Tehran, Iran

Background: Vitamin D (Vit D), as an immunomodulator, has been hypothesized to play a critical role in the pathogenesis of endometriosis. Thus, in this study, we evaluated whether there is an association between 25-hydroxyvitamin D [25(OH)D] and susceptibility to endometriosis in Iranian women.

Methods: Women of reproductive age, including 56 healthy women and 54 patients with endometriosis, were enrolled in the study. Serum levels of 25(OH)D, calcium, parathyroid hormone (PTH), and peritoneal fluid (PF) levels of 25(OH)D were assessed.

Results: The serum and PF levels of 25(OH) D in the patients with endometriosis were significantly lower than in the control group (p=0.001 and p= 0.03, respectively). Subjects with serum levels of 25(OH)D lower than 20 ng/mL had a 2.7 times higher risk of endometriosis than people with 25(OH)D serum levels higher than 20 ng/mL (non-deficient) (OR = 2.7, 95 % confidence interval: 1.24−5.80, p=0.01). The serum levels of calcium and PTH were significantly lower and higher in patients with endometriosis compared with controls, respectively (p<0.001, p=0.02, respectively). Also, the serum levels of 25(OH)D were lower in stages I-II endometriosis than in stage III-IV; however, no significant difference was observed.

Conclusion: Our findings showed that people with Vit D deficiency are at higher risk of endometriosis.

Keywords: 1, 25(OH)$_2$ D$_3$, Endometrial stromal cells, Endometriosis

Stem Cells and Immune Cell Therapy

A2ar/Tim3-Double KD-MSLN-CAR T Cells Effectively Kill Tumor Cells in Vitro and in Vivo

Tahereh Soltantoyes[1], *Behnia Akbari*[1], *Zahra Shahossein*[2], *Hamid Reza Mirzaei*[1], *Jamshid Hadjati*[1]

1. Tehran University of Medical Sciences, Tehran, Iran.
2. Iran University of Medical Sciences, Tehran, Iran.

Background: The immunosuppressive tumor microenvironment in solid tumors increase the expression of inhibitory receptors including Tim3 and A2aR on CAR T cells. These inhibitory receptors, Tim3 and A2aR, attach to their ligands on the surface of CAR T cells and decrease their antitumor effectiveness. Here, we examined the impact of simultaneous shRNA-mediated knockdown of Tim3 and A2aR expression in CAR T cells in vitro and in vivo.

Methods: Using standard molecular methods, MSLN-CAR T, Tim3.KD. MSLN-CAR T, A2aR.KD. MSLN-CAR T, and Tim3/A2aR.KD.MSLN-CAR T cells were produced. Cytotoxicity, Cytokine production, and proliferation of CAR T cells were evaluated in the presence and absence of adenosine in coculture with HeLa cells. The in vivo function of each group of CAR T cells was examined through measurements of tumor volume and mice survival.

Results: Our findings demonstrated that MSLN-CAR T cells were able to significantly kill tumors in vitro and stabilize tumor growth in vivo. However, mice injected with MSLN-CAR T cells died unexpectedly early through the experiments. Interestingly, Tim3/A2aR.KD.MSLN-CAR T cells showed enhanced antitumor potency both in vitro and in vivo compared to MSLN-CAR T cells. Furthermore, although both A2aR.KD. MSLN-CAR T cells and Tim3/A2aR.KD.MSLN-CAR T cells retained their antitumor ability in vitro, only Tim3/A2aR.KD.MSLN-CAR T cells significantly slowed down the tumor growth rate in vivo.

Conclusion: The rapid mortality of mice in the MSLN-CAR T group may be caused by lymphoproliferation. Although functional in vitro, single KD. MSLN-CAR T cells failed to control tumor growth in vivo due to the additional functions of these receptors. Since the downregulation of A2aR promotes the cytotoxic function and proliferation of CAR T cells in vitro, it can be assumed that reducing the expression of the inhibitory receptors in an optimum setting can be a potential strategy for the treatment of solid tumors in clinical studies.

Keywords: CAR T, mesothelin, Adaptive cell therapy, Tim3, A2aR, shRNA

Altered Immune Responses in Mice after Receiving Nicotine-Pulsed Mesenchymal Stem Cell-Conditioned Medium

Leila Mahmoudzadeh[1], *Seyyed Meysam Abtahi Froushani*[1], *Rahim Hobbenaghi*[1], *Alireza Mahmoudian*[1], *Vahid Mohammadi*[2]

1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Department of Internal Diseases and clinical pathology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Background: Previous investigations have documented that nicotine-pulsed mesenchymal stem cells (MSCs) can induce an anti-inflammatory phenotype in some immune cells in vitro. This study aimed to assess the effects of nicotine-pulsed MSCS On the function of immune cells, macrophages, and lymphocytes of mice receiving these cells.

Methods: Bone marrow-derived MSCs (1.5×106) were seeded in a T75flask and incubated with 0, .1, .5, or 1 µM nicotine until the cells reached 90% confluency. Afterward, immunophenotyping change, vitality, the concentration of TGF-β, IL-10, and IDO levels of the MSC-conditioned medium were examined. Correspondent to in vitro results, the C57BL/6 mice intravenously received 400 µL of the conditioned medium of MSCs (CM), conditioned medium of nicotine (.5 µM)-pulsed MSCs (CMN), or medium. After 12 h, the lymphocytes, neutrophils, and peritoneal macrophages of the mice were isolated and their function was evaluated ex vivo. The least effective dose concentration of nicotine that led to an anti-inflammatory environment by the MSC-conditioned medium was 0.5 µM.

Results: Nicotine at this concentration prompted a higher level of TGF-β, IDO concentration in the conditioned medium. However, this concentration did not affect the MScs' markers expressions or MScs' vitality. T lymphocytes isolated from the mice receiving CMN showed a significant decrease in proliferation rate. The ratio of the IFN-γ gene expression to IL-4 gene expression in splenocytes was significantly reduced in the mice receiving CMN compared to the mice receiving CM.

Conclusion: The neutral red uptake, respiratory burst, and nitric oxide production of the peritoneal macrophage only decreased in the mice treated with CMN. These factors also decreased in neutrophils isolated from mice receiving CM or CMN. However, these decreases were more prominent in the mice treated with CMN.

Keywords: Nicotine, Mesenchymal stem cells, Immunomodulation

Assessment of Extracellular Vesicles from Rabbit Ear Pina Cells

Fateme Govahi[1], Maryam M. Matin[1], Azadeh Haghighitalab[1], Ahmadreza Bahrami[1]

1. Department of Biology Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

Background: In recent decades, stem cell technology has been among the most interesting topics. Despite their potential, their disadvantages have drifted researchers' attention towards the great potentials of cellular secretome components, e. g. extracellular vesicles (EVs). Rabbit ear pina is known for its fast regeneration upon injury. Previous studies have shown that cells derived from the same tissues are indicating stemness properties and they can contribute to regeneration in the same way as their tissue of origin.

Methods: In this study cells from rabbit ears were cultured in exosome-depleted serum upon reaching proper confluency, and their conditioned medium (CM) was collected. For the isolation of EVs from precleared CM, we used ultracentrifuge in 110.000g for 2h in 4^{o}C as the gold standard method. Total protein contents were measured by the BCA assay and EVs were characterized by particle size analysis, zeta potential analysis, and atomic force microscopy (AFM).

Results: The results obtained from BCA assay indicate efficient EV isolation regarding their protein content. Particle size analysis shows that the EVs cover a range from 30 to 150 nm in diameter. Moreover, zeta potential analysis confirm that the EV preparations have good colloidal stability. The images of AFM also confirms their proper integrity and spherical morphology.

Conclusion: Blastema cells can secret the EVs and we could isolate them.

Keywords: Rabbit Ear Pina Cells, Secretome, Extracellular Vesicles

Blocking the STAT3 Signaling Pathway Leads to Producing Fully Mature Dendritic Cells That Increase the Expression of The ID2 Transcription Factor

Maryam Ajami[1], *Seyed Mohammad Moazzeni*[1]

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background: It was reported that the STAT3 gene deletion in the dendritic cells (DCs) vaccine is associated with a lower frequency of Treg and increased amounts of IFNγ+ T cells in the TIL population. Cytokines and their subsequent signal transducers can alter the DCs functions through specific transcription factors. ID2 is a transcriptional mediator, which controls DCs lineage diversification by STAT-dependent pathways. In this study, we investigated the effect of inhibiting the STAT3 signaling pathway on the induction of human dendritic cells.

Methods: After isolating monocyte cells from peripheral blood, and differentiation of immature DCs, R848 as an inhibitor of STAT3 was added to the DCs culture along with the maturation factor. The western blot method was used to examine the level of ID2 expression. DCs surface markers, antigen uptake, and inflammatory cytokine production were used to determine the level of maturation.

Results: The western blot result showed that the expression of ID2 increased significantly following DCs treatment with STAT3 inhibitor. The significant increase in the expression of maturation surface markers and the amount of inflammatory cytokine secretion in STAT3-blocked dendritic cells confirmed the higher maturity state of this group compared to the control group. Decreasing the FITC-Dextran intake by R848 treated DCs, also confirmed the maturity of these cells.

Conclusion: Collectively, blocking of the STAT3 pathway, through increasing the expression of transcription factor ID-2, causes an increase in the maturation state of dendritic cells. Considering the importance of DCs maturation state in the induction of an appropriate immune response, it can be concluded that inhibition of STAT3 should potentiate the efficacy of DC vaccines by providing a strong co-stimulation signal and production of inflammatory cytokines.

Keywords: Fully Matured Dendritic Cell, STAT3 signaling pathway, ID2 transcription factor, Phagocytosis, Maturation Marker, Inflammatory Cytokine

Chitosan-Embedded hTERT-MSCs-IDO1-EVs Reduce Blood Leukocytes after Spinal Cord Injury

Shiva Amanollahi[1], Ahmad Reza Bahrami[2,3], Azadeh Haghighitalab[3], Hanieh Shaterzadeh- Yazdi[4], Hossein Kazemi-Mehrjerdi[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran
2. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran
3. Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran
4. Ph.D. of veterinary pathology, Private veterinarian, Mashhad, Khorasan Razavi, Iran

Background: Reducing the migration of leukocytes after secondary damage in the lesion site helps to preserve the spinal cord tissue and improves movement ability. Although their initial penetration rate is increased within 48 to 72 hours and may continue for up to 7 days, their presence and activity should be reduced over time. Due to the ability of nano-sized MSCs-EVs for passing through blood-brain barrier and their immunomodulatory properties, they may be effective to modulate leukocyte infiltration following spinal cord injury (SCI).

Methods: In the present study, hTERT-immortalized human adipose tissue-derived mesenchymal stem cells were used, in which the ectopic expression of the human IDO1 gene is stably increased. As a control group, hTERT-MSCs-GFP were applied. To isolate extracellular vesicles (EVs), 72-hour conditioned media were collected under EV-depleted culture conditions. Following the pre-clearing steps, the extraction of small EVs (sEVs) was performed based on the ultrafiltration method. Freshly prepared sEVs were mixed with modified chitosan hydrogel under sterile conditions and were kept on ice. Female rats (N=60, ~230 g) were used and divided into five groups including Sham, SCI, Hydrogel, hTERT-MSCs-GFP-EVs, and hTERT-MSCs-IDO1-EVs. Laminectomy of the T10 was performed based on the previously described method. The drake aneurysm clip is placed on the spinal cord for 1 minute. 60 µl of hydrogel or hydrogels containing 100 µg sEVs from control GFP and IDO1 groups were locally injected immediately after SCI. After 72 h and 8 weeks, blood samples were collected and Giemsa staining was done to determine the differential leukocyte counts.

Results: No significant differences were observed between the groups 72 hours after SCI (p>0.05). In the first 3 days, the highest monocyte counts were reported for IDO1 (4.5±1.52) and GFP –EVs (4.33±1.63) groups. 8 weeks later, monocytes were remarkably decreased in IDO1 (1.78± 1.48) and GFP –EVs (1.83± 1.60) groups. These changes were statistically significant in comparison to the hydrogel (4.67± 1.37) and sham (4.17± 1.94) groups. In addition, MSCs-IDO1-EVs reduced the lymphocyte infiltration in comparison to other groups (8 weeks).

Conclusion: The administration of EVs, enriched from the conditioned media of genetically modified MSCs, in the benefit of IDO1, may be an effective approach to managing the inflammatory status and reducing tissue damage following the SCI.

Keywords: Spinal cord injury, Mesenchymal stem cells, Small extracellular vesicles, Indoleamine 2, 3, -dioxygenase, Leukocytes

Combinational Administration of Mesenchymal Stem Cell-Derived Exosomes and Metformin Reduces Apoptosis in an In Vitro Model of Insulin Resistance in Hepg2 Cells

Kosar Malekpour[1], Ali Akbar Pourfathollah[2]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Immunology, Faculty of medical sciences, Tarbiat Modares University, Tehran, Iran

Background: In the pathogenesis of liver damage related to type 2 diabetes, inflammation-mediated apoptosis of liver cells, including hepatocytes, is crucial. Also, inflammation and producing inflammatory cytokines by affecting hepatic stellate cells (HSCs) leads to excessive activity. Activated HSCs produce a large amount of different extracellular matrix components and lead to liver fibrosis. Therefore, controlling the inflammation that damages the liver in type 2 diabetes is very important. For this reason, in this study, we have used metformin and mesenchymal stem cells derived exosomes (with proven anti-inflammatory effects) to reduce inflammatory responses and apoptosis of hepatocytes.

Methods: Insulin is added to the high glucose culture medium of HepG2 cells to induce insulin resistance in them. Metformin and mesenchymal stem cell exosomes (MSC-EXO) were used alone and in combination to treat these cells. After isolating mesenchymal stem cells from Wharton's jelly tissue, these cells were evaluated for differentiation and expression of specific surface markers. Then, their supernatant was collected after adapting them with a 0% FBS medium to isolate exosomes from these cells. The amount of total protein was checked using the BCA total protein assay. To check survival and apoptosis, MTT and Annexin/PI tests were used. Due to the effect of metformin on gluconeogenesis and glucose uptake in liver cells, the supernatant of each well was collected, and glucose content was detected using a glucose assay kit (glucose oxidase method).

Results: The results showed that glucose consumption in the metformin-treated (3.161 ± 0.23 mmol/L, $p<0.001$) and combination therapy groups (3.451 ± 0.2 mmol/L, $p<0.001$) were significantly increased compared to the HG DMEM and insulin-treated groups. However, MSC-EXO alone has no significant effect on glucose consumption compared to HG-treated HepG2 cells (1.392 ± 0.13 mmol/L, $p=0.8516$). The results of the MTT test showed that the viability of HepG2 cells in the combination therapy by metformin and MSC-EXO was higher than the other experimental groups (82.54 ± 1.31, $p<0.001$). Also, the apoptosis rate in the combination therapy group was associated with a 46.321 % decrease compared to the HG DMEM and insulin treatment group, which is statistically significant ($p=0.0396$).

Conclusion: The results show that the combined treatment of metformin and MSC-EXO with a higher ability can prevent the apoptosis of HepG2 cells. Also, the consumption of glucose was higher in the treatment group. Therefore, it can be concluded that the simultaneous use of metformin and MSC-EXO is a better treatment than their individual use.

Keywords: Mesenchymal stem cell, Exosome, Diabetes, Insulin resistance, Inflammation, Immunomodulation.

Combinational Administration of Mesenchymal Stem Cell-Derived Exosomes and Metformin Reduces Inflammatory Responses in an in Vitro Model of Insulin Resistance in Hepg2 Cells

Kosar Malekpour[1], Ali Akbar Pourfathollah[2]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Immunology, Faculty of medical sciences, Tarbiat Modares University, Tehran, Iran

Background: Exosomes are 30-150 nm endosome-derived vesicles that secrets from all cell types. These nano-vesicles carry cellular lipids, proteins, mRNA, and non-coding RNA and deliver their contents to the target cells differently. Recently, exosomes have been considered drug delivery systems. Also, the combinational administration of exosomes alongside different drugs can increase the drug's efficacy.

Methods: Human Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) and HepG2 cell lines were cultured in T75 culture flasks to reach 80% confluency. Then the cells were washed with PBS and adapted to culture with FBS-free DMEM/F12 culture medium for 3 days. Finally, the exosome was isolated using the precipitation method. Size and morphology of exosomes determined by DLS and SEM analysis. The exosome protein content of collected samples was estimated by BCA assay. To evaluate the immunomodulatory effects of MSC-EXO, metformin, and their combinatory uses on HepG2 cells, these cells are cultured in 6 plate wells. Then the cytokines (IL-1β, IL-6, TNF-α, and IL-10) expression and production are evaluated using qRT-PCR and ELISA, respectively.

Results: The results show that the expression and production of inflammatory cytokines, including IL-1β, IL-6, and TNF-α, in the combined treatment receiving groups show a more significant decrease than those receiving single treatment. Also, these results show that the expression and production level of IL-10 as an anti-inflammatory cytokine has increased in the group that received the combined treatment.

Conclusion: Since inflammatory cytokines play a vital role in the pathogenesis of liver-related diseases in type 2 diabetes, controlling inflammation is very important. Therefore, according to the results of this study, it can be said that the combined treatment of metformin and MSC-EXO by involving different pathways can be a better treatment than each alone.

Keywords: Mesenchymal stem cell, Exosome, Diabetes, Insulin resistance, Inflammation, Immunomodulation.

Comparison of the Modulated Effects of Tretinoin and Calcitriol Treated Mesenchymal Stem Cell Supernatant on Macrophage Functions

Majdedin Ghalavand[1], Samira Mohammadi-Yeganeh[2], Seyed Mahmoud Hashemi[3], Hadi Esmaeili Gouvarchin Ghaleh[1]

1. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Medical Nanotechnology and Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: According to several studies, calcitriol and tretinoin can regulate differentiation as well as the growth of mesenchymal stem cells (MSCs). Nevertheless, the relationship between the supernatant of macrophage and mesenchymal stem cells is still under investigation. In the present work, a comparison is made between the modulated impacts of calcitriol and tretinoin-treated mesenchymal stem cell supernatant on macrophage functions.

Methods: The isolation of mesenchymal stem cells was done using mouse bone marrow and the various concentrations of calcitriol (200 and 400 nM) and tretinoin (25, 50, and 100 nM) were used to pulse MSCs for 48 h. Macrophages were then applied to co-culture the supernatant of MSCs for 4 hr. Consequently, macrophages were assessed for respiratory burst.

Results: Based on the obtained results, supernatant of bone marrow-derived MSCs pulsed with calcitriol and tretinoin can have the potential for decreasing the respiratory burst of macrophages considerably in comparison with the control group.

Conclusion: The anti-inflammatory M2 macrophage polarization can be accelerated using calcitriol and tretinoin by mesenchymal stem cells. Keywords: Tretinoin, Calcitriol, Macrophage, Mesenchymal stem cell supernatant.

Keywords: Tretinoin, Calcitriol, Macrophage, Mesenchymal stem cell supernatant

Different Stages of Lupus Disease and Mesenchymal Stem/Stromal Cells Transplantation Outcome

Akram Hoseinzadeh[1], Zahra Rezaieyazdi[2], Mahmoud Mahmoudi[1], Jalil Tavakol Afshari[1], Fahimeh Lavi Arab[1], Seyed-Alireza Esmaeili[1]

1. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Rheumatology, Ghaem Hospital, Mashhad University of Medical Science, Mashhad, Iran

Background: Will the results of mesenchymal stem cell transplantation (MSCs) be the same in different stages of lupus disease?

Methods: We used the Pristane-induced lupus (PIL) mice model as an autoinflammatory disease. MSCs transplantation was performed and then the serum level of TGF-β, IL-17, and the percentage of Treg/Th17 cell subsets in splenocytes, respectively by enzyme-linked immunosorbent assay (ELISA) and flow cytometry analysis, were analyzed and compared. Experiments were carried out with different initiation treatment time points (early and late stages of disease). Analysis of variance (ANOVA) followed by post hoc Tukey's test was used for multiple comparisons.

Results: At the end of the study, a significant decrease in TGF-β levels along with a significant increase in IL-17 was shown in PIL mice compared to normal mice. However, MSCs transplantation manifested a dual role in the outcome: increased TGF-β serum level and decreased IL-17 level in the treatment strategy in the late stages (L-MSCs) of the disease, while the opposite in the treatment strategy in the early stages (E-MSCs) of the disease.

Conclusion: The obtained results, enhance our understanding of the immunoregulatory function of MSCs in different cytokine microenvironments.

Keywords: Mesenchymal stem cells, Immunomodulation, Systemic lupus erythematosus, Pristane.

Dose-Dependent Protective Effects of Azithromycin (AZT) and Extracellular Vesicles Derived from Mesenchymal Stem Cells (MSC-Evs) on Biochemical Parameters in the Cecal Ligation and Puncture (CLP) Model of Sepsis

Fatemeh Ahangari[1], Sara Soufi[2], Zahra Mirsanei[1], Sara Soudi[3], Seyed Mahmoud Hashemi[1]

1. Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran.
2. Tabriz University of Medical Sciences, Tabriz, Iran.
3. Tarbiat Modarres University (TMU), Tehran, Iran.

Background: Both azithromycin (AZT) and extracellular vesicles derived from mesenchymal stem cells (MSC-EVs) have protective effects on vital organs against sepsis. This study evaluated a combinational therapy consisting of AZT (with three doses) and MSC-EVs on biochemical parameters, including alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine to determine which dose can be more effective.

Methods: C57BL/6 female mice were randomly divided into the sham group, the cecal ligation and puncture (CLP) group, the AZT group, the MSC-EVs group, and the AZT+MSC-EVs group. Azithromycin was injected in 3 doses of 30, 50, and 100 mg/kg in treatment groups alone or groups with MSC-EVs. The CLP group underwent abdominal surgery and received sterile saline via intravenous injection (i.v.), the AZT group received 30, 50, or 100 mg/kg AZT via intraperitoneal injection (i.p.), the MSC-EVs group received 200 mg/kg of MSC-EVs via i.v. injection and the AZT+MSC-EVs group received i.p. injection of 30, 50, or 100 mg/kg of AZT and a tail vein injection of 200 mg/kg of MSC-EVs. After 24 hours, mice were euthanized by exsanguination under anesthesia. The serum was separated from blood clots and biochemical parameters, including ALT, AST, BUN, and creatinine were measured.

Results: MSC-EVs treatment combined with AZT (30 mg/kg) showed a significant reduction in the BUN and creatinine levels ($p<0.05$); however, there is no significant reduction in AST and ALT levels. In comparison with AZT (30 mg/kg), combinational therapy with AZT (50 mg/kg) can only significantly reduce creatinine levels ($p<0.05$) with no effects on other biochemical parameters. Moreover, AZT (100 mg/kg) in combination MSC-EVs can merely reduce BUN and AST levels significantly ($p<0.05$).

Conclusion: Combinational therapy of AZT (30 mg/kg) and MSC-EVs (200 mg/kg) can be more effective in decreasing biochemical parameters and organ protection rather than higher doses of AZT (50 and 100 mg/kg). This is maybe due to the reduction of possible adverse effects of high doses of AZT, specifically on the liver. Keywords: Extracellular Vesicles, Exosomes, Mesenchymal stem cells, Sepsis.

Keywords: Extracellular Vesicles, Exosomes, Mesenchymal stem cells, Sepsis

Engineering Chimeric Autoantibody Receptor T Cells for Targeted B cell Depletion in Multiple Sclerosis Model: An in-Vitro Study

Maryam Sahlolbei[1], Jafar Kiani[1], Seyed Mahmoud Hashemi[2], aref shiralipour[2]

1. Iran University of Medical Sciences (IUMS), Tehran, Iran.
2. Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran.

Background: Recent evidence suggests that B cells and autoantibodies have a substantial role in the pathogenesis of Multiple sclerosis (MS). Myelin basic protein (MBP) is an autoantigen, antibodies against which target the myelin components of the central nervous system. To specifically deplete MBP-reactive B cells, T cells could be engineered to express chimeric autoantibody receptors (CAARs), which have an epitope of MBP in their extracellular domain acting as bait for trapping autoreactive B cells. This study aims to assess the function of the designed MBP-CAAR T cells against autoreactive B cells extracted from the experimental autoimmune encephalomyelitis (EAE) mice model.

Methods: In vitro, T cells were transduced to express a CAAR consisting of MBP as the extracellular domain and the CD137 and CD3zeta as the cytoplasmic domains. The experimental autoimmune encephalomyelitis (EAE) was induced by injecting MBP (amino-acid: 83-95) into C57BL/J mice. B cells were isolated and co-cultured with the engineered T cells. The cytotoxicity and cytokine production of the MBP-CAAR T cells was investigated and compared with Mock T cell (empty vector transduced T cell) and Un-T cells (un-transduced T cell) at 1:1, 5:1, and 10:1 ratio.

Results: MBP-CAAR T cells showed higher cytotoxic activity against autoreactive B cells in all effector-to-target ratios compared to Mock T cells (empty vector-transduced T cell) and Un-T cells (un-transduced T cell). CAAR T cells proliferated and released inflammatory cytokines in CAAR T: target co-culture, higher than Un-T and Mock T cell groups. Our data demonstrated that T cells expressing MBP-CAAR construct potently kill autoreactive B cells and release interleukin-2 (IL-2), Tumor necrosis factor-α (TNF- α), and Interferon- γ (IFN-γ) in vitro.

Conclusion: Based on these findings, CAAR T cells are promising for curing or modulating autoimmunity and can be served as a new approach for clone-specific B cell depletion therapy in multiple sclerosis.

Keywords: Autoreactive B cells, Chimeric Autoantibody Receptor (CAAR), Experimental Autoimmune Encephalomyelitis (EAE), Multiple Sclerosis (MS)

Engineering Human Wharton's Jelly Mesenchymal Stem Cells Derived- Exosomes to Encapsulate Mir 124, Effectively Inhibits Hepatic Stellate Cell Proliferation, Apoptosis, and Cell Cycle

Bahare Niknam[1], Kaveh Baghaei[2], Davar Amani[1], Seyed Mahmoud Hashemi[1]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Liver fibrosis is a common pathological process of liver diseases. The activation of hepatic stellate cells (HSCs) is currently a major cause of liver fibrosis, and inducing HSC apoptosis is indispensable for reversing it. Due to the evidence that miR-124 levels are aberrant in various liver diseases, we investigated whether delivery of miR-124 through exosomes derived from human Wharton's jelly mesenchymal stem cells (hWJMSC-Exo) could increase the apoptosis of HSCs in vitro.

Methods: Supernatant of Human Wharton's jelly mesenchymal stem cells cultured cells gathered after exposure to serum-free media. Exosomes isolated by commercial kit and characterized by dynamic light scattering, transmission and scanning electron microscopy, and bicinchoninic acid assay. Modified CaCl2 method applied for miR-124 loading in exosomes (ExomiR) and loading confirmation assessed by the relative expression of miR-124. A viability test was performed on LX-2 cells treated with either Exos or ExomiR in multiplicity concentrations. Moreover, the apoptosis rate of the cells and the cell cycle were evaluated.

Results: Isolated exosomes were imaged by TEM and SEM. Exosomes displayed round, oval-shaped, and lipid-bilayer membrane-bound structures. The majority of exosomes were in a range of 70–100 nm. miR-124 expression significantly enhanced in ExomiR-124 compared to Exo. This data confirmed the successful enrichment of Exosomes by miR-124. The viability of LX-2 cells was harnessed following the treatment with ExomiR in a time–dose–dependent manner and ExomiR-124 could induce apoptosis and arrest the cell cycle at G0/G1 phase.

Conclusion: Exosomes isolated from the hWJMSCs were found to have the potential to deliver miR-124 into hepatic stellate cells properly with high functionality maintenance for miR-124 in case of inhibiting the proliferation and promoting the apoptosis of HSCs.

Keywords: miR-124, Exosomes, Liver fibrosis, Apoptosis

Epigallocatechin-3-Gallate Augments Therapeutic Effect of Bone-Marrow-Derived Mesenchymal Stem Cells in Experimental Autoimmune Type-1 Diabetes

Fatemeh Ahmadzadeh[1,2], Saeid Abedian[1,2]

1. Department of Immunology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Immunogenetics Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: Type 1 diabetes stem-cell-based therapy is one of the most promising therapeutic strategies for pancreatic damage treatment due to regenerative and immunomodulatory functions. However, previous studies have demonstrated that the high glucose concentration or diabetic environment suppresses some crucial proteins and increases senescence in stem cells. Green tea epigallocatechin gallate (EGCG), a known antioxidant and anti-inflammatory agent, has proven benefits on cell protection. This study was designed to assess the effect of mesenchymal stem cell (MSC) administration and its combination with EGCG on the differentiation of T helper subsets and pancreatic regeneration.

Methods: MSCs were extracted from the bone marrow of normal mice and cultured. Type 1 diabetes was induced in C57BL/6 by multiple injections of streptozotocin (STZ). The diabetic groups were treated intraperitoneally with PBS, MSCs, EGCG, and a combination of MSCs and EGCG. Thereafter, the gene expression of T-bet and GATA-3 and the serum level of IFN-γ, IL-4, and insulin were evaluated using real-time PCR and ELISA, respectively. Histopathology was used for the assessment of pancreatic islets using HE staining.

Results: Investigating the cross-effect of MSCs and EGCG on immune response shift, results showed combinational treatment modulated T-bet and GATA-3 expression and restored Th1/Th2 balance more efficiently. Reduced production of IFN-γ as a proinflammatory cytokine and increased secretion of IL-4 as a regulatory cytokine was also shown. Moreover, MSCs and EGCG monotherapy were associated with enhanced serum insulin levels and recovered pancreatic islet morphology, but combined MSCs and EGCG treatment were more effective.

Conclusion: Taken together, combined therapy with EGCG and MSCs transplantation show clinical potential in type 1 diabetes treatment through synergetic effects in maintaining Th1/Th2 balance and the regeneration of damaged pancreatic tissues.

Keywords: Mesenchymal Stem Cell, Epigallocatechin gallate, Type 1 diabetes

Evaluation Of Expression of Placenta-Specific Molecule 1 (PLAC-1) in Enriched Cancer Stem-Like Cells Derived from Invasive Human Prostate Cancer Cell Line (PC-3) in Comparison with Tumor Parent Cell Population

Pooya Farhangnia[1], Roya Ghods[2], Reza Falak[1], Amir-Hassan Zarnani[3], Ali-Akbar Delbandi[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: During the last two decades, immunotherapeutic approaches have revolutionized the treatment of cancers. However, a major obstacle in cancer immunotherapy is the identification of tumor-specific antigens to achieve targeted therapy with less damage to healthy cells. Placenta-specific molecule 1 (PLAC-1) is one of the recently discovered cancer testis antigens, and there is accumulating evidence that PLAC-1 is activated in various types of tumors, playing a role in malignancy and cancer progression. In the current study, the expression of the PLAC-1 molecule was investigated for the first time on prostate cancer stem cells (PCSCs) derived from the PC-3 human prostate cancer cell line, and its expression was compared with the population of tumor parent cells at the gene and protein levels.

Methods: PCSCs were enriched using the three-dimensional cell culture method or sphere formation assay. The expression of genes related to stemness and pluripotency, including SOX2, OCT4, Nanog, C-Myc, and KLF-4, and the expression of stem cell differentiation molecules, including CD44 and CD133 were evaluated in the enriched cells and tumor parent cells by Real-time PCR and flow cytometry, respectively. Finally, the expression of the PLAC-1 molecule in enriched cells and tumor parent cells was evaluated at the gene and protein levels by real-time PCR and flow cytometry.

Results: PC-3 tumor cells formed spheroids composed of cancer stem cells in a non-adherent medium. The expression of SOX2, OCT4, Nanog, C-Myc genes ($p< 0.01$) and the expression of CD44 and CD133 molecules ($p<0.05$) were significantly higher in PCSCs than in parent cells. However, this increased expression was not significant for the KLF-4 gene. The expression of the PLAC-1 molecule in PCSCs was significantly increased compared to parent cells in both genes ($p<0.01$) and protein ($p<0.001$) levels.

Conclusion: In conclusion, we have demonstrated for the first time that PLAC-1 is up-regulated in PCSCs. We propose that PLAC-1 may serve as a potential target for immunotherapeutic approaches targeting PCSCs.

Keywords: PLAC-1, Cancer stem cell, Immunotherapy, Cancer testis antigen

Evaluation of the Proliferative and Anti-Apoptotic Potential of Nano-Silymarin on Mesenchymal Stem Cells

Farid Zeynali[1], Fahime Lavi[2]

1. Department of Urology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Immunology and Allergy School of Medicine University of Mashhad, Mashhad Iran

Background: Silymarin is obtained from Silybum marianum seed, an ancient herb with anti-inflammatory, anti-oxidant properties but several limitations such as poor bioavailability and little solubility have restricted its successful translation in a clinical setting. The nano-micelle delivery system is a highly reproducible method with a versatile platform capable to improve poor-water solubility and bioavailability of free-SL. Mesenchymal stem cells (MSCs) are multipotent cells proficient to regenerate lost tissues. MSCs have similar properties to SL including immunomodulatory, and anti-oxidant effects. There is no study to guide the effects of nano-SL on MSCs.

Methods: In this study, the MTT proliferative test and anti-apoptotic test by anexin/PI were performed. Effects of different concentrations (1, 2.5, 5, and 10 µM) of nano-micelle-SL on MSCs derived from adipose tissue (AT)-MSCs were investigated.

Results: Our findings indicated that nano-SL (5 and 10 µM) encourages adipose-derived (AD)-MSCs proliferation and protects from apoptosis. Conclusion: The current study was an in vitro work aimed to investigate whether co-treatment of nano-SL could be a potent combinatory therapy.

Conclusion: Our finding indicated survival and repair-promoting activities of MSCs were improved in the presence of nano-SL and the beneficial effects of each of nano-SL and MSCs can be reinforced when mixed. These promising preclinical results can be applied to clinical practice

Keywords: Nano-silymarin, Mesenchymal stem cells, Apoptosis

Evaluation of the Anti-Inflammatory Effects of Mesenchymal Stem Cells Treated with Naloxone on the Clinical Appearance and Histopathology of Experimental Ulcerative Colitis

Mozhdeh damirchi[1], Hadi Esmaeili Gouvarchinghaleh[1], Mahdieh Farzanehpour[1]

1. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Background: Ulcerative colitis is an inflammatory bowel disease that can be diagnosed with secretory disorders. The use of drugs for treatment, in addition to chemical side effects, imposes exorbitant costs on patients. Today, various treatment methods have been proposed, such as stem cell therapy. The aim of this study was to determine the effect of Naloxone-treated mesenchymal stem cells in the treatment of ulcerative colitis.

Methods: Colitis was induced by acetic acid in four groups of rats and one group was considered as a group of healthy control (negative control) rats. In post-induction treatment groups, one control patient group received no drug (positive control), one group was treated with two million Naloxone-treated mesenchymal stem cells, one group was treated with two million untreated mesenchymal stem cells alone, and one group was treated with Mesalazine. After 10 days, rats were evaluated for fecal status, production of inflammatory mediators, and production of inflammatory cytokines.

Results: The results showed that mesenchymal stem cells alone and in combination with Naloxone significantly reduced the production of inflammatory mediators and cytokines compared to the positive control group ($p<0.05$).

Conclusion: Overall, the use of Naloxone with mesenchymal stem cells appears to reduce the symptoms of ulcerative colitis in our experimental model. Due to the ease of isolation and rapid growth of mesenchymal stem cells, it can be used as an adjunctive therapy to improve the condition of patients with ulcerative colitis, which requires more molecular research.

Keywords: Mesenchymal stem cells, Colitis, Naloxone

Evaluation of the Effect of Mesenchymal Stem Cells' Conditioned Medium on the Gene Expression of CCL3 and CCL4 Chemokines in the Spinal Cord of Rats with Neuropathic Pain

Mohammad-Shafi Mojadadi[1], Samad Nazemi[2], Kimia Shahrazad[3]

1. Department of Immunology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.
2. Department of Physiology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran.
3. Student Research Committee, Sabzevar University of Medical Sciences, Sabzevar, Iran.

Background: Chronic nerve pain and neuropathy are among the most annoying types of pain following trauma, inflammation, cancer, diabetes, autoimmune diseases, AIDS, and herpes. In neuroinflammation, activation of a network of cytokines-chemokines may play an important role in the development of neuropathic pain. In this study, we tried to investigate the possible effects of mesenchymal stem cells (MSCs)-derived conditioned medium (CM) on the expression of CCL3 and CCL4 chemokines in the spinal cord of rats with neuropathic pain induced by chronic constriction injury (CCI) surgery.

Methods: After the isolation of MSCs from the bone marrow of Wistar rats and culturing them in the laboratory, the CM was collected in the second passage. In the next step, CM was injected intraperitoneally into the rats of the experimental group on three different days (the day before CCI surgery and the 7th and 11th days after surgery). Rats in the control group received DMEM medium during this period. Anti-neuropathic pain effect of MSCs-CM was evaluated using a behavioral hot plate, and von Frey tests on days -1, 3, 6, 9, 12, and 15 of the CCI surgery. In addition, at the end of the experiment (day 15), the expression of CCL3 and CCL4 chemokine genes in the spinal cord of the animals was measured by Real time-PCR technique.

Results: The results obtained from the behavioral tests showed that CM could significantly reduce thermal hyperalgesia and mechanical allodynia in the test group compared with the control group. Moreover, molecular studies showed that the expression of CCL3 in the group receiving the test group was significantly lower than in the CCI group, and CCL4 was unchanged.

Conclusion: Intraperitoneal injection of CM to CCI model of neuropathic pain can significantly reduce pain. It seems that anti-neuropathic pain effect of CM may be partly mediated by reducing CCL3 chemokine in the spinal cord.

Keywords: Neuropathic pain, hyperalgesia, allodynia, mesenchymal stem cell conditioned medium, Chemokine, CCL3, CCL4

Evaluation of the Immunomodulatory Properties of Chitosan-Embedded hTERT-MSCs-IDO1-Evs after Spinal Cord Injury

Shiva Amanollahi[1], Ahmad Reza Bahrami[2,3], Azadeh Haghighitalab[3], Hanieh Shaterzadeh-Yazdi[4], Hossein Kazemi Mehrjerdi[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran
2. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran
3. Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran
4. Ph.D. of veterinary pathology, Private veterinarian, Mashhad, Khorasan Razavi, Iran

Background: Spinal cord injury (SCI) is a destructive complication that leads to temporary or permanent paralysis of a person or animal. The increase in inflammation and the release of pro-inflammatory cytokines at the site of the lesion worsen the condition. Treatment goals should be aimed at reducing or modulating these factors. Indoleamine 2, 3- dioxygenase (IDO1) is one of the main paracrine factors of mesenchymal stem cells (MSCs). It was demonstrated that the soluble factors and extracellular vesicles (EVs) from the conditioned medium of MSCs may mimic their producer cells' immunomodulatory properties. EVs could be administered as hydrogel-embedded entities to enhance their local persistence in the damaged tissue.

Methods: In the present study, hTERT-immortalized human adipose tissue-derived mesenchymal stem cells were used, in which the ectopic expression of the human IDO1 gene is stably increased. As a control group, hTERT-MSCs-GFP were applied. To isolate extracellular vesicles (EVs), 72-hour conditioned media were collected under EV-depleted culture conditions. Following the pre-clearing steps, the extraction of small EVs (sEVs) was performed based on the ultrafiltration method. Freshly prepared sEVs were mixed with modified chitosan hydrogel under sterile conditions and were kept on ice. Female rats (N=30, ~230 g) were used and divided into five groups including Sham, SCI, Hydrogel, hTERT-MSCs-GFP-EVs, and hTERT-MSCs-IDO1-EVs. Laminectomy of the T10 was performed based on the previously described method. The drake aneurysm clip is placed on the spinal cord for a minute. 60 µl of hydrogel or hydrogels containing 100 µg sEVs from control GFP and IDO1 groups were locally injected immediately after SCI. 72 h after surgery, the rats were euthanized and central and epicenter parts of the lesion were removed, and placed in RIPA solution. The levels of interleukin (IL)-6, IL-10, and tumor necrosis factor alpha (TNF-α) were measured in the supernatant of spinal cord homogenates using ELISA kits.

Results: Comparison of the concentration of different cytokines among the experimental groups reveals that the changes in the level of TNF-α was significant (p<0.05) for IDO1 (43.11±8.21) vs. control groups (54.21±6.21). Moreover, the ratio of IL-6/IL-10 (0.14±0.05) and TNF-α/IL-10 (0.23±0.08) were decreased for MSCs-IDO1-EVs, indicating their anti-inflammatory effects in comparison to other treatment groups, although it was not statistically significant.

Conclusion: MSCs-EVs enriched from the conditioned medium of the cells equipped with the ectopic expression of IDO1, carry immunomodulatory content which may be effective in the treatment of inflammatory diseases.

Keywords: Spinal cord injury, Mesenchymal stem cells, Small extracellular vesicles, Indoleamine 2, 3, -dioxygenase, Cytokine measurement

Evaluation of the Effect of Intranasal Injection of Differentiated Rat MSCs with Tolerogenic Probiotics in Modulating the Behavior and Immune Response of the Parkinson's Rat Model

Negar Ebadpour[1], Seyed-Alireza Esmaeili[1], Mahmoud Mahmoudi[1]

1. Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Parkinson's disease (PD) is considered a common progressive neurodegenerative disease in the world. Inflammation in the substantia nigra pars compacta (SN) and other brain areas damage dopaminergic cells that lead to Parkinson's disease. Research has proven that bone marrow mesenchymal stem cells (MSC) with their anti-inflammatory, neuroprotective, and antioxidative properties can effectively prevent and reduce the symptoms of PD disease. Recent reports suggested that probiotics with tolerogenic properties can have a significant anti-inflammatory effect in patients with PD. Therefore, combining both approaches can be effective as an ideal tool for Parkinson's treatment. This study examines the effects of intranasal injection of differentiated rat MSCs in combination with tolerogenic probiotics on the behavior and immune response of 6-OHDA Parkinson's rats.

Methods: Forty male Wistar rats (6-8 weeks) were divided into 5 groups including sham, positive control (received levodopa after 6-OHDA injection), negative control (received normal saline), treatment I (received intranasally MSCs) and treatment II (received intranasally MSCs incubated with probiotics) for 4 weeks. Finally, apomorphine-induced rotational tests, open-field, hanging, and rotarod as well as the expression levels of IL-1β, IL-6, TNF-α, IL-10, TGF-β, and α-Synuclein, evaluated by Real-Time PCR methods. Plus, the amount of ROS evaluated by the ROS kite.

Results: The study is in process and the data will be presented in the Congress.

Conclusion: Upon the neuroinflammatory responses that occur in the Parkinson's model and the anti-inflammatory properties of MSCs and probiotics we expect that these combination cell therapies can show a proper anti-inflammatory or immunoregulatory response in the inflamed area in the brain 6-OHDA rat. Therefore, we anticipate the treatment can affect the results of behavioral tests and the oxidative stress level. Overall, the study can be provided comprehensive information for a better understanding of MSCs features and the effects of probiotics on these features as well as new ways to prevent and treat Parkinson's disease.

Keywords: Parkinson's disease, Mesenchymal Stem Cell, 6-OHDA rat, Probiotic, Cytokine

Evaluation of the Effects of Tetrandrine on Antioxidant and Anti-Inflammatory Properties of Adipose-Derived Mesenchymal Stem Cells

Shohreh Fadaghi[1], Merat Mahmoodi[1], Mahdi Ranjkesh[1], Fatemeh Mozafari Khosravi[2]

1. Department of Immunology, School of medicine, Kerman University of Medical sciences
2. School of medicine, Kerman University of Medical sciences

Background: Mesenchymal stem cells (MSCs) have immunomodulatory and anti-inflammatory effects, and adipose tissue is a very good source for isolating these cells. The functional flexibility and phenotype polarity of MSCs are one of the most important reasons for the failure of treatment with MSCs. One of the best ways to increase the therapeutic properties of MSCs before entering the body is to pre-treatment them in the culture medium with plant extracts. Tetrandrine (TET) is a plant alkaloid that has anti-inflammatory, immunomodulatory, and antioxidant properties. Therefore, we evaluated the effect of tetrandrine (1μM) on the viability and proliferation, antioxidant, and anti-inflammatory properties of adipose-derived MSCs (AD MSCs) in vitro.

Methods: MSCs were purchased from the cell bank of Afzalipour at Kerman University of medical sciences. Then, cells were cultured in a 48-well plate, and treated with TET (1 μM) for 48 hours next to the control group (non-treated). finally, the MTT test was performed to evaluate viability and proliferation of MSCs, kynurenine level was measured spectrophotometrically to measure IDO enzyme activity, antioxidant tests (SOD3, TAC, and MDA) were performed using Randox and Naxifer kits, And ELISA test was performed to measure the level of TGF-B cytokine.

Results: The proliferation and viability of TET-treated groups were not different from the control group. The amount of kynurenine secreted from the treated MSC was significantly higher than the control group. The amount of SOD3 and TAC increased significantly compared to the control group after treatment with TET, but the amount of MDA had no significant difference with the control group. And the amount of TGF-B secretion after treatment with TET was not significantly different from the control group.

Conclusion: Our results showed that tetrandrine increases the anti-inflammatory (through increasing SOD3 and IDO activity) and antioxidant potentials of MSCs without any adverse effects on their cell viability and proliferation.

Keywords: Mesenchymal stem cells (MSCs), Tetrandrine, Viability, Anti-inflammatory

Examining the Level of Inflammatory Cytokines TNF-A and IL.8 Produced by Osteoblasts Differentiated from Dental Pulp Stem Cells

Hastar[1], Mandana Sattari[1]

1. Department of Immunology. School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The use of dental pulp stem cells (DPSCs) in clinical applications instead of bone marrow stem cells is a very promising method capable of significantly changing the future of medical treatment. If further studies prove that DPSCs and the cells differentiated from them do not stimulate the immune system, these cells can be used more reliably in treatment.

Methods: In this research, we studied the isolated DPSCs and differentiated osteoblasts in a medium without inflammatory stimulants in terms of TLR3 and TLR4 gene expression and inflammatory cytokines TNF-α and IL.8 and measured the concentration of inflammatory cytokines IL.8 and TNF.α produced by these two types of cells.

Conlusion: The obtained results showed that the expression level of inflammatory cytokines IL.8 and TNF.α in differentiated osteoblasts is significantly different as compared with DPSCs. However, no significant difference was observed in TLR.4 expression between the two groups. An increase in TNF.α expression level was found to directly correlate with an increase in the expression of IL.8. The concentration of cytokine TNF-α in osteoblasts was measured to be greater than that of IL.8 in DPSCs.

Conclusion: In comparison to DPSCs, osteoblast cells first lead to inflammatory responses. These responses reduce over time. However, DPSCs retain their immunomodulatory properties and do not show inflammatory responses.

Keywords: Dental pulp stem cells (DPSCs), Osteoblast, Differentiated, Inflammatory cytokine, Toll-like receptor

Human Adipose-Derived Mesenchymal Stem Cell Exosomes Attenuated the Induced-Experimental Autoimmune Encephalomyelitis, a Chronic Model of Multiple Sclerosis

Morteza Jafarinia[1], Fereshteh Alsahebfosoul[2], Hossein Salehi[3], Nahid Eskandari[2], Maryam Azimzadeh[4], Merat Mahmoudi[5], Sedigheh Asgary[6], Mazdak Ganjalikhani Hakemi[2]

1. Department of Immunology, School of Medicine, Kerman University of medical sciences
2. School of medicine, Kerman University of Medical sciences
1. Shiraz Neuroscience Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Anatomical Sciences, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
4. Khomein University of Medical Sciences, Khomein, Iran.
5. Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
6. Isfahan Cardiovascular Research Centre, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran

Background: In recent years, mesenchymal stem cells (MSCs)-derived exosome (Exo) have been widely studied in inflammatory diseases, as these vesicles have not the cell therapy's concerns, such as the chance of malignancy, genetic disability, and immune rejection. In the current study, we investigated the therapeutic effects of human adipose-derive mesenchymal stem cells (hADSC)-Exo on experimental autoimmune encephalomyelitis (EAE) and compared it with hADSCs and control mice.

Methods: C57Bl/6 mice were treated intravenously with hADSCs, hADSC-Exos, or vehicle (PBS) after EAE induction. Every other day, the weight and clinical score of each mouse were noted. On day 30, mice were sacrificed, and splenocytes were extracted for use in a proliferation assay and a flow cytometry analysis to determine the frequency of T regulatory (Treg) cells. Also, the spinal cord was used for assessing leukocyte infiltration by hematoxylin and eosin (H&E) staining, percentages of demyelination areas by luxol fast blue (LFB) staining, and mean fluorescence intensity (MFI) of oligodendrocyte transcription factor 2 (OLIG2) and myelin basic protein (MBP) by immunohistochemistry.

Results: Our findings demonstrated that treating with hADSC- and hADSC-Exo significantly decreased the maximum mean clinical score (MMCS), myelin oligodendrocyte glycoprotein (MOG)-induced proliferation of splenocytes, inflammation score, and the percentages of demyelination areas in comparison to mice treated with PBS ($p<0.05$). Our results also showed that hADSC-treated mice have a higher frequency of CD4+CD25+Foxp3+ cells compared to control mice ($p=0.023$). No significant difference was seen in the MFI of MBP and OLIG2 in the spinal cord of the studied groups.

Conclusions: Overall, we propose that intravenous treatment of hADSC- Exo reduces T cell proliferation potential, mean clinical score, leukocyte infiltration, and demyelination in a chronic MS model, thereby attenuating the induced EAE.

Keywords: Experimental autoimmune encephalomyelitis, Mesenchymal stem cells, exosomes, Multiple sclerosis, Adipose tissue, human adipose-derive mesenchymal stem cells.

Human Amniotic Epithelial Cells Exert Anti-Cancer Effects through Secretion of Immunomodulatory Small Extracellular Vesicles (Sev)

Mohammad-Reza Bolouri[1], Roya Ghods[2], Kayhan Zarnani[3], Sedigheh Vafaei[4], Reza Falak[1], Amir-Hassan Zarnani[3]

1. Department of Immunology, Iran University of Medical Sciences, Tehran, Iran
2. Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4. Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Background: We identified here the mechanism by which hAECs exert their anti-cancer effects.

Methods: We showed that vaccination with live hAEC conferred effective protection against murine colon cancer and melanoma but not against breast cancer in an orthotopic cancer cell inoculation model.

Results: hAEC induced strong cross-reactive antibody response to CT26 cells, but not against B16F10 and 4T1 cells. Neither heterotopic injection of tumor cells in AEC-vaccinated mice nor vaccination with hAEC lysate conferred protection against melanoma or colon cancer. Nano-sized AEC-derived small-extracellular vesicles (sEV) (AD-sEV) induced apoptosis in CT26 cells and inhibited their proliferation. Co-administration of AD-sEV with tumor cells substantially inhibited tumor development and increased CTL responses in vaccinated mice. AD-sEV triggered the Warburg's effect leading to Arginine consumption and cancer cell apoptosis. Our results clearly showed that it is AD-sEV but not the cross-reactive immune responses against tumor cells that mediate the inhibitory effects of hAEC on cancer development.

Conclusion: Our results highlight the potential anti-cancer effects of extracellular vesicles derived from hAEC.

Keywords: Stem cells, Human amniotic epithelial cell, Cancer, small extracellular vesicles (sEV), Cytotoxic T lymphocyte, Warburg's effect, metabolomics

Immunomodulatory Effects of Calcitriol on Mesenchymal Stem Cell-Derived Adipose Tissue

Nafise Daneshi[1], soolmaz kheradmand[1], niayesh salar[1], fateme mohammdi[1], fahimeh lavi[1], Mahmoud mahmoudi[1]

1. Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Immunology Research Center, Bu-Ali Research Institute, Faculty of Medicine, Mashhad

Background: Multiple sclerosis (MS) is a progressive neurodegenerative inflammatory disease. One pathological feature of MS is to irreversibly damage the myelin shielding of neurons in the central nervous system (CNS). It is considered that the loss of self-tolerance is involved in the pathogenesis of MS. Studies showed that the active form of 1,25-dihydroxycholecalciferol (Calcitriol) and mesenchymal stem cells (MSCs) have immunomodulatory effects on the immune system and could be helpful for regenerative therapeutic application in neurodegenerative diseases.

Methods: The MSCs were isolated from adipose tissue and characterized using surface CD markers pattern and differentiation capacity into adipose, osteocyte, and chondrocyte lineages. After that, the confirmed MSCs were cultured in the presence of different concentrations of 1,25-dihydroxycholecalciferol (6, 12, 25, 50, 100 nM, and 0 as control). Regulatory T cells (Tregs) population analysis using flow cytometry techniques.

Results: Our findings indicated that 50 and 100 nM concentrations of 1,25-dihydroxycholecalciferol significantly increased AT-MSCs expanded Tregs proportion.

Conclusions: This study demonstrated that 1,25-dihydroxycholecalciferol has an effective role in the AT-MSCs by inducing regulation. The main conclusion of this study is that 1,25-dihydroxy vitamin D3 can act as a beneficial synergistic factor in the treatment of patients undergoing stem cell therapy.

Keywords: Immunomodulatory, Calcitriol, Mesenchymal stem cell,

In Vitro Study: Evaluation of the Effect of Lupus Serum in the Period of Relapse and Remission Phase on Survival of the Umbilical Cord-Derived Mesenchymal Stem Cells

Zahra Baridjavadi[1], *Mahmoud Mahmoudi*[1], *Seyed-Alireza Esmaeili*[1]

1. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Systemic lupus erythematosus (SLE), is a systemic autoimmune disease, with a variable clinical course that targets a wide range of organs. Common treatments include a variety of, occasionally serious adverse effects, and some patients are non-responsive or resistant to all therapies. As a result, there is an increasing need to develop new therapeutic methods. Recently, mesenchymal stem cells (MSCs) have been extensively studied as a promising option in the treatment of several autoimmune diseases, including SLE. Although various studies support the effectiveness of MSC transplantation, the conflicting results require further studies to determine whether the disease activity of SLE reduces the suppressive capacity of MSCs or not. In this study, we evaluate the effect of lupus serum in different SLE phases on MSCs.

Methods: Serum of 12 Lupus patients (6 in the relapse phase and 6 in the remission phase) and 6 healthy controls incubated with MSCs. The frequency of apoptotic cells of treated different SLE phase serum in comparison with healthy controls was estimated. The expression level of complement inhibitor genes “before and after exposure” was measured by the Real-Time PCR method.

Results: The study is in process and the data will be presented in the Congress.

Conclusion: Stem cell transplantation is one of the emerging treatment candidates for the treatment of lupus patients. Patients with different levels of autoantibodies, complement, and other serum components “in different stages of the disease” can have destructive or apoptotic effects on cells. Therefore, investigation of the effect of lupus patient’s serum at different phases on MSCs functions and survival can be helpful to a better understanding of when is the best time for the patients to receive MSCs as treatment.

Keywords: Systemic lupus erythematosus, Mesenchymal Stem Cell, Apoptosis

In Vitro, Treatment of Murine Splenocyte with Mesenchymal Stem Cell-Derived Extracellular Vesicles Altered the mRNA Levels of Master Regulators Gene of T cell Subsets

Alireza Yeganeh[1], Anwar Fathollahi[2], Seyed Mahmoud Hashemi[2], Farshid Yeganeh[2]

1. Department of Biology, Faculty of science, University of Guilan, Rasht, Iran
2. Department of Medical Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The aim of the present study was to investigate the effect of extracellular vesicles derived from mesenchymal stem cells on the expression of the master regulator genes corresponding to subsets of T helper cells and the production of related cytokines by them.

Materials and methods: The mesenchymal stem cells (MSCs) were isolated from the abdominal adipose tissue of C57BL/6 mice; then extracellular vesicles were extracted from the conditioned media of the second passage of MSCs. Splenocytes (106???) of healthy mice were activated using anti-CD3 and anti-CD28 antibodies, then treated with MSC-EVs. The proliferation rate of spleen cells and the frequency of Treg cells were investigated using flow cytometry. In addition, the expressions of T helper cell subset-specific transcription factors were evaluated using a real-time PCR assay. The effect of MSC-EVs on the production of IFN-γ, IL-17a, IL-10, and TGF-β cytokines by splenocytes was evaluated using ELISA.

Results: The results showed that the treatment of CD3/CD28-activated-splenocytes with MSC-EV did not change their proliferation rate. However, after the treatment, the mRNA levels of FoxP3 and Elf4 as well as the frequency of regulatory T cells were significantly increased in comparison to the control group. Although the expression levels of master regulators i.e. Gata3, Rorc, and Tbx21 were down-regulated, the levels of the corresponding cytokines (IL-10, IL-17, and IFN-y) were not changed.

Conclusion: The results showed that MSC-EVs were capable to increase the frequency of CD4+CD25+Foxp3+ T cells and increasing the Foxp3 mRNA level in splenocytes.

Keywords: Mesenchymal stem cell, Extracellular vesicles, Immunomodulation

Induced Pluripotent Stem Cells Drive LKB1-AMPK Signaling to Attenuate Pulmonary Fibrosis

Marjan Taherian[1], Paria Bayati[1], Nazanin Mojtabavi[1]

1. Department of Immunology, faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Pulmonary fibrosis (PF) is a heterogeneous lung disease characterized by aberrant production of extracellular matrix (ECM) and irreversible scar formation leading to respiratory failure and death. Effective therapy is still absent, and lung transplantation is the only available curative intervention. Idiopathic pulmonary fibrosis (IPF) is a more common and severe form of PF with a median survival of 2–5 years after diagnosis. Recently, induced pluripotent stem cells (iPSCs) have been suggested for the treatment of PF; however, the mechanism of their anti-fibrotic function is poorly understood.

Methods: iPSCs were generated by a lentiviral vector to deliver the four pluripotency genes into mouse embryonic fibroblasts to reprogram them back to the pluripotent state. The experimental model of IPF induced by surgical instillation of bleomycin (BLM) into the trachea, 48 h after, stem cell treatment was performed on the BLM-challenged mice, through injection of iPSCs into the tail vein. Lung fibrosis was evaluated by histologic examinations during Hematoxylin and eosin and Masson-Trichrome staining, as well as hydroxyproline measurement within extracted lung specimen, besides evaluating the wet/dry lung weight ratio and percent of animal weight lost. Moreover, investigating the gene expression was conducted by qPCR. iPSC transplantation significantly reduced the body weight lost in BLM-received animals, and also, attenuated the inflammatory characteristics, ECM deposition and, hydroxyproline content within their lung tissues.

Results: Treatment with iPSCs upregulated the BLM-inhibited expression of low-density lipoprotein receptor related protein-1 (Lrp1), liver kinase B1 (Lkb1), Adenosine monophosphate-activated protein kinase (Ampk), and Unc-51 like autophagy activating kinase 1 (Ulk1) genes, and suppressed BLM-induced expression of Connective tissue growth factor (Ctgf), and Autophagy-related protein 13 (Atg13) genes.

Conclusion: Our finding implicated an anti-fibrotic effect for low-density lipoprotein receptor related protein-1 (LRP1) and LKB1-AMPK signaling, by which iPSCs ameliorate pulmonary fibrosis.

Keywords: induced pluripotent stem cells (iPSCs), fibrosis, pulmonary fibrosis, fibrotic signaling, LKB1-AMPK signaling, Autophagy

Induced Pluripotent Stem Cells Inhibit Bleomycin (BLM)-Induced Pulmonary Fibrosis in Mice via Suppressing TGF-B1 Induced MTOR Signaling

Marjan Taherian[1], Paria Bayati[1], Nazanin Mojtabavi[1]

1. Department of Immunology, faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Idiopathic pulmonary fibrosis (IPF) is a chronic disease with a high mortality rate and no effective therapy. Over the last decade, induced pluripotent stem cells (iPSCs) have emerged as a novel resource for stem cell-based therapeutic approaches for hard-to-cure disorders. Although their anti-fibrotic mechanisms are obscure, recent evidence implicated the therapeutic potential of iPSCs in IPF. Hence, the present study aimed to investigate the impact of iPSCs on the several mediators and signaling molecules, which are contributed to IPF pathogenesis.

Methods: Lentiviral plasmids were applied for reprogramming of mouse embryonic fibroblasts to generate iPSCs, upon transferring Oct4, Sox2, Klf4, and c-Myc. An intratracheal administration of BLM was conducted to induce pulmonary fibrosis in C57/Bl6 mice. Then, iPSCs were intravenously injected into the half of BLM-received mice. 21 days following BLM instillation, pulmonary fibrosis was evaluated through histological examinations and hydroxyproline assay. The expression of target genes was investigated by qPCR. Treatment with iPSCs remarkably ameliorated the pathological features of lung fibrosis, including inflammation, extracellular matrix deposition, and collagen content. Furthermore, iPSC significantly inhibited BLM-induced upregulation of Transforming growth factor beta 1 (TGF-β1), Platelet-derived growth factor (PDGF), and Platelet-derived growth factor receptor (PDGFR) genes, and BLM-induced BLM-suppressed expression of Vascular endothelial growth factor-A (VGF-a) gene.

Results: In addition, iPSC therapy significantly reversed the BLM-induced overexpression of Ras homolog enriched in the brain (Rheb), Raptor, Ribosomal protein S6 kinase beta-1 (S6k1), Eukaryotic translation initiation factor 4E-binding protein 1 (4ebp1), Zeb, and Snail genes, as well as down-regulation of Tuberous sclerosis 2 (Tsc2) and back to near-normal levels observed in the control group. This study indicated that iPSC transplantation ameliorates the IPF partly by modulating the fibrosis-related mediators, and suppressing the mammalian target of rapamycin (mTOR) signaling during lung fibrosis.

Conclusion: Our results shed light on the new anti-fibrotic mechanisms of the iPSCs that could be translated into clinical settings.

Keywords: induced pluripotent stem cells (iPSCs), fibrosis, TGF-β signaling, mTOR pathway

Innovative Manufacture of NK Cells from Umbilical Cord Blood Mononuclear Cells

Marzieh Ebrahimi[1], Parisa Shams[2], Delbar Daneshjou[3], Yasaman Noori[3]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Iran
2. Cell and Developmental Biology Department, Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, Iran
3. Research and Development Department, Kian Immune Cell Co. (KIC), Iran

Background: To reach "off-the-shelf "NK products which can be cost-effective and avoid a difficult purification process, the umbilical cord blood mononuclear cells (UCB-MNCs) could be a suitable, feasible, and available source. Cord blood stem cells are thought to be an efficient approach for clinical-grade manufacturing the NK cells which have great self-renewal, proliferative, and hematopoiesis capacities and can effectively differentiate into many distinct cells of the erythroid, lymphoid, and myeloid lineages. Therefore, in the present study, we aim to manufacture NK cells from umbilical cord blood mononuclear cells without purification and feeder-free.

Methods: Umbilical cord blood (UCB) units were collected in cord blood bags after written informed consent with regard to scientific use from the cord blood bank of the Royan Stem Cell Technology Company (RSCT Co., Iran). UCB-MNCs were harvested from healthy umbilical cord blood by Ficoll-Hypaque density gradient centrifugation and were cultured in cell culture plates with RPMI1640 medium, 5% autologous serum in the presence of the cytokine cocktail, small molecule inhibitors, and immunosuppressive drug for effective differentiation of NK cells from HSC/HPCs without purification and feeder-free for 21 days. After 21 days NK cells' viability, fold increase, purity, and cytotoxicity against U-251 cells were analyzed.

Results: Using flow cytometry based-assays, we showed expansion and differentiation of HSC/HPCs to CD56+CD3- cells with a mean 31-fold increase, 40% purity, and >90% viability without purification and feeder-free. In addition, our final product represents no T-cell contamination and about 40% cytotoxicity against U-251 cells.

Conclusion: This platform has advantages over existing procedures, as it allows easier, time-saving, and cost-effective manufacturing of NK cells from umbilical cord blood mononuclear cells.

Keywords: Natural killer cells, Umbilical cord blood, Cancer immunotherapy, Stem cell research

Investigating the Immunomodulatory Effects of Manipulated Mesenchymal Stem Cells with IDO1 Gene and Their Small Extracellular Vesicles in the Repair of Heart Damage in MI Rat Model

Alireza Karimi[1], Maryam M. Matin[1,2,3], Azadeh Haghighitalab[1], Hossein Kazemi Mehrjerdi[4], Masoud Rajabioun[4], AhmadReza Bahrami[1]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran
2. Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
4. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad

Background: The treatment method based on the use of stem cells to improve the symptoms of patients with heart attacks was first reported in 2001. Since then, the use of stem cells from different sources in patients with cardiovascular diseases is being considered from time to time. In addition to their ability to differentiate, MSCs also have the capacity to modulate the function of the immune system, which has made them an attractive therapeutic tool. In recent decades, the importance of innate and acquired immune responses in the control of myocardial function has been seriously discussed during health and diseases. It has been shown that dysregulation of the immune system can lead to the induction of local devastating immune responses and trigger inflammatory events causing serious side effects associated with acute MI. IDO1 is a key enzyme in the tryptophan catabolism pathway. The immunomodulatory role of this enzyme has been reported in several studies and conditions, including pregnancy, chronic infection, autoimmune diseases, organ transplantation, and drug resistance in a variety of cancers.

Methods: A rat model of heart attack was created based on the Cryo method and hTRET-IDO1 cells and their Exosomes were injected into the desired heart area. Histological examinations and immune cells examination were performed by CBC test and differential white blood cell count. Repair of heart damage was also evaluated by echocardiography on the day before surgery plus the days 1, 28, and 55 post surgery followed by a TTC test.

Results: In this study, it was shown that by modulating the immune system and reducing inflammation by cells and Exosomes, carrying a higher level of IDO1, in the damaged heart the repair rate is significantly increased.

Conclusion: Inflammation has been considered a key factor for the development of complicated diseases such as atherosclerosis, the creation of arterial platelets and delay in tissue repair during heart attack. It has been shown that targeting inflammatory pathways can effectively reduce atherosclerosis in animal models of cardiovascular diseases. In the conditions of inflammation, the presence of pro-inflammatory cytokines is essential for the activation and calling of functional cells of the immune system and the regeneration of damaged tissue. The problem appears when the level of pro-inflammatory cytokines continuously increases and rises above the appropriate level. In this case, instead of calling progenitor cells to the damaged tissues, autoimmune responses are created, and inflammatory mechanisms destroy the tissue structure. So by modulating the immune system, it is possible to increase cardiac function.

Keywords: Mesenchymal Stem Cells, Heart Damage, IDO1, MI Rat Model, Modulation of the Immune System

Investigation of Immunoregulatory Impact of Lupus Mesenchymal Stromal Cells in TCD4+ Subsets and Their Secreted Cytokines of Healthy and Lupus Balb/C Mice Models

Sara Hosseini[1], Seyed-Alireza Esmaeili[1], Mahmoud Mahmoudi[1], Maryam Rastin[2], Abbas Shapouri Moghadam[3], Akram Hosseinzadeh[1], Nafiseh Tabasi[2], Mahdieh Khazaee[2], Zahra Javanmardi[1]

1. Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Immunology Research Center, Bu-Ali Research Institute, Medical School, Mashhad University of Medical Sciences, Mashhad, Iran
2. Immunology Research Center, Bu-Ali Research Institute, Medical School, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Immunology, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Systemic Lupus Erythematosus is a complex autoimmune disease with unknown etiopathogenesis. Various studies have considered SLE as a result of stem cell disorder. Allogeneic MSC transplantation ameliorates disease activity while there is ambiguity in using the lupus individual's MSCs due to the patient's cell source. The aim of this in vivo study was to compare the effects of the immunoregulatory properties of MSCs derived from lupus and healthy mice on the TCD4 + cell subsets and their secreted cytokines in the serum of Balb/c mice models.

Methods: SLE disease was induced in the female Balb/c aged 4 weeks by injection of pristane and the healthy group received PBS. The body weight and clinical characteristics of mice were measured in two-month intervals. After 6 months, the mice were sacrificed, and BM-MSCs were isolated. Then, lupus and healthy MSCs were injected into experimental groups. The mice were euthanized and splenocytes were isolated. The frequency of TCD4+ cells (Th1, Th2, Th17, and Treg) from the spleen was measured by flow cytometry. Furthermore, the serum cytokine levels of IFN-γ, IL-4, IL-17, and TGF-β were measured by ELISA.

Results: Our flow cytometry results demonstrated that the injection of lupus MSCs into healthy and lupus mice led to a further rise in frequencies of the inflammatory Th1, Th2, and Th17 cells. Moreover, consistent with the L-MSC injection, the serum levels of IFN-γ, IL-4, and IL-17 cytokines were also augmented. In addition, the Treg cell population in lupus mice receiving L-MSCs compared to the control groups was elevated although serum levels of TGF-β didn’t increase after L-MSC injection in mice.

Conclusion: Lupus mesenchymal stem cells have immunomodulatory defects and may also play a role in disease progression due to their lupus source. Thus, they might not propose the most suitable source of MSC transplantation and it is better to perform autologous MSC transplantation with more caution.

Keywords: Systemic lupus erythematosus, Mesenchymal stromal cells, Th1, Th2, Th17, Treg, IFN-γ, IL-4, IL-17, TGF-β

Investigation of the Effect of IFN-Γ/TNF-A-Treated Mesenchymal Stem Cells on Th9- And Treg Cell-Related Parameters in a Mouse Model of Ovalbumin-Induced Allergic Asthma

Abdollah Jafarzadeh[1], Parvin Nozari[2]

Background: Th9- and regulatory T (Treg) cells exert pro- and anti-allergic activity, respectively. Mesenchymal stem cell (MSC)-related immunomodulatory impacts can be enhanced by inflammatory cytokines. Here, the modulatory effects of IFN-γ/TNF-α-induced MSCs on Th9- and Treg cell-related parameters were investigated using an asthma model.

Methods: Allergic asthma was induced in BALB/c mice using sensitized and challenging with ovalbumin (OVA). The asthmatic groups were treated intraperitoneally with PBS, MSCs, IFN-γ-induced MSCs, TNF-α-induced MSCs, and 'IFN-γ + TNF-α'-induced MSCs before the challenge phase. The mice were sacrificed 24 h after the challenge. The serum IL-9 and IL-35 levels, as well as gene expression of IL-9, PU.1, IL-35-EBI3, and FOXP3 in the lung tissues, were assessed using ELISA and real time-PCR, respectively.

Results: The differences in Th9 and Treg-related parameters were not significant between untreated asthmatic mice and those treated with non-induced MSCs. In comparison with the untreated asthmatic group, treatment with IFN-γ-induced MSCs significantly reduced serum IL-9 levels, and reduced lung expression of IL-9 and PU.1, while increasing serum IL-35 levels as well as lung expression of FOXP3; treatment with TNF-α-induced MSCs significantly reduced serum IL-9 levels as well as lung expression of IL-9 and treatment with 'IFN-γ + TNF-α'-induced MSCs, significantly modulated all investigated Th9 and Treg-related parameters.

Conclusion: In comparison to mice treated with non-induced MSCs, serum IL-9 levels were remarkably decreased in mice treated with IFN-γ-induced and 'IFN-γ + TNF-α'-induced MSCs. IFN-γ-and 'IFN-γ + TNF-α' treated MSCs exerted almost comparable impacts, but were more efficient than TNF-α-exposed MSCs. Thus, IFN-γ alone can be sufficient to promote the immunomodulatory effects of MSCs.

Keywords: Allergic asthma

Macrophage Phenotypes Modulation by Cell Shape-Imprinted PDMS

Mohammad Majidi[1], Saeedreza Pakzad[2], Maryam Salimi[3], Abdolnaser Azadbakht[4], Shahin Bonakdar[5], Mazaher Gholipourmalekabadi[1]

1. Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Food and Drug Laboratory Research Center, Food and Drug Administration, Iran Ministry of Health and Medical Education, Tehran, Iran
3. Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Biomedical Engineering, Central Tehran Branch, Islamic Azad University, Tehran, Iran
5. National Cell Bank, Pasteur Institute of Iran, P.O. Box 13169-43551, Tehran, Iran

Background: Differentiation is tightly regulated by two major signaling pathways, growth and mechanical factors. The topographically patterned material surface can alter the shape and function of the cells. Polydimethylsiloxane (PDMS) is a non-toxic and very inexpensive material that is widely used for cell-to-cell contact studies. In this study, we investigated the effects of different macrophage (MQ)-imprinted PDMS stamps on the behaviors of RAW264.7 monocyte cell line (MO).

Methods: Light, SEM, and florescent microscopy, AFM analysis, LAL assay, cell viability assay, Real-time PCR, ELISA, Cytokine assay, and Flow Cytometry were used to evaluate the effects of PDMS stamps on the behaviors of MO.

Results: The gene expression profiles and flow cytometry of MQ revealed that although the cell shape microstructure promotes MQ phenotypes according to the specific shape of each pattern gene expression and CD markers are not identical with so far realized MQ subtypes. The ELISA results were in agreement with the gene expression profiles (Table 1).

Conclusion: In conclusion, the wound dressings with surface topography of M2 might induce M2 Anti-inflammatory responses and accelerate wound healing with minimal hypertrophic scar formation, although further pre-clinical and clinical experiments should be carried out to prove this claim.

Keywords: Macrophage, Topography, Cell-imprinting, wound dressing, Anti-inflammatory

Mesenchymal Stem Cell-Based Therapy for Chronic Wound Healing; Systematic Review

Soheil Sadr[1], Mahsa Khiyabani[1], Hossein Kazemi Mehrjerdi[1]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: The skin can be damaged by wounds caused by cutting or breaking of the tissue and burns. As a result of their secreted factors with anti-inflammatory, anti-fibrotic, and pro-angiogenic activities, mesenchymal stem cells (MSCs) are able to improve wound healing in a number of ways. These factors can either be expressed encapsulated inside membrane vesicles (microparticles and exosomes) or in soluble molecules (growth factors, cytokines). To summarize how mesenchymal stem cells influence wound healing and describe the latest approaches to enhance their therapeutic value in non-healing wounds, this review aims to summarize the mechanisms underlying that effect.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on Mesenchymal Stem Cell-Based therapy for chronic wound healing from January 2000 to January 2023. Fifteen affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: MSCs can act indirectly through the secretion of immunomodulatory and bioactive factors. Several growth factors produce growth factors that stimulate cell proliferation and survival, including VEGF, HGF, and leukemia inhibitory factors (LIF). MSCs also produce interleukin (IL)-6 and 10 as well as prostaglandin E-2 (PGE-2), transforming growth factor (TGF), or nitric oxide (NO). As a result, they inhibit T lymphocyte proliferation, inhibit T lysis, prevent NK cell activation and macrophage activation, and modulate B cell proliferation, among other immunomodulating effects.

Conclusion: In conclusion, mesenchymal stem cells (MSCs) are able to improve wound healing, and they secrete factors that have anti-inflammatory, antifibrotic, and pro-angiogenic functions, which have been shown to play a significant role in wound healing.

Keywords: Mesenchymal stem cells, Skin regeneration, wound healing, Systematic review

Modulation of the PI3K/AKT Pathway Genes by Induced Pluripotent Stem Cells in the Context of Experimental Mouse Idiopathic Pulmonary Fibrosis

Paria Bayati[1,2], Marjan Taherian[1,2], Hadi Poormoghim[3], Nazanin Mojtabavi[1,2]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
3. Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran

Background: Of the novel approaches showing promising results in the preliminary in vivo studies of idiopathic pulmonary fibrosis are induced pluripotent stem cells. Paving their way through clinical application necessitates discovering their full mechanism of action; here we demonstrate their contribution to the modulation of the PI3K/AKT pathway as one of the most prominent non-SMAD pathways downstream of the TGF-β signaling.

Methods: lentiviral vectors carrying four pluripotency factors (OCT4, SOX2, KLF, and c-MYC) were utilized for induction of the iPS cells from mouse embryonic fibroblasts. Mice (age ≈ 8 weeks) were randomly allocated in Control, Bleomycin, and bleomycin+iPS groups; experimental IPF was induced by surgical instillation of bleomycin into the lungs of mice and the control group received PBS instead. 2 days post bleomycin administration, the bleomycin+iPS mice were injected with iPS cells via the tail vein rout. After histological examinations, the expression of the genes for PI3K, AKT, PDK1, RICTOR, GSK3β, FOXO1, PTEN, PPARγ, and SNAIL1 was assessed by specific primers and SYBR green qPCR.

Results: Statistical analyses revealed that after the bleomycin exposure, PI3K, AKT, PDK1, RICTOR, and PPARγ, as well as SNAIL were upregulated, while GSK3β, FOXO1, and PTEN were downregulated in comparison with the control group. The expression profile of the corresponding genes in the BLM+iPS group was shown to be very similar to the control healthy mice.

Conclusion: For the first time we have demonstrated that the iPS cells not only affect the TGFβ induced SMAD signaling but also are capable of modulating the PI3K/AKT pathway, thereby alleviating inflammation and fibrotic responses in the lungs of IPF mouse models. This finding adds significant insight to the current knowledge about the mechanism of action of the iPS cells which could be expanded into other fibrotic or inflammatory disorders, also it is a forward step in their clinical applications.

Keywords: PI3K, AKT, GSK3b, FOXO, IPSCs, Idiopathic pulmonary fibrosis, PTEN, PPARγ

Ninety-Six–Hour Starved Peripheral Blood Mononuclear Cell Supernatant Inhibited LA7 Breast Cancer Stem Cells Induced Tumor via Reduction in Angiogenesis and Alternations in Gch1 and Spr Expressions

Maryam Mehri[1], Reza Gheitasi[2], Roghayeh Pourbagher[3], Mohammad Ranaee[4], Kosar Nayeri[1], Seyed Mostafa Rahimi[1], Hamid Reza Khorasani[5], Hadi Hossein-Nattaj[6], Davood Sabour[7], Haleh Akhavan-Niaki[3], Sadegh Fattahi[8], Behnam Kalali[9], Amrollah Mostafazadeh[3]

1. Student Research Committee, Babol University of Medical Sciences, Babol, Iran
2. Institute for Infectious Diseases and Infection Control, Jena University Hospital, Jena, Germany
3. Cellular & Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
4. Department of Pathology, Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran.
5. Department of Cancer Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Isar, Babol, Iran
6. Immunology Department, Mazandaran University of Medical Sciences, Sari, Iran
7. Department of Genetics, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran
8. North Research Center, Pasteur Institute of Iran, Amol, Iran
9. Department of Medicine II, Klinikum Grosshadern, LMU University, Munich, Germany

Background: The microenvironment of solid tumors is heterogeneous, containing different cells, namely, cancer stem cells and immune cells. We previously reported the immunoregulatory behavior of the human immune cell in a solid tumor microenvironment-like culture under serum starvation stress for 96h.

Methods: Ninety-six–hour starved PBMC supernatant (96 h-SPS) was collected after culturing human PBMCs for 96h under serum starvation conditions. Breast cancer stem cells, LA7 cell line, was used for in vitro study by analyzing gene expression and cytotoxicity, proliferation, scratch wound healing assays, followed by in vivo tumor induction in three groups of mature female Sprague Dawley rats. Animals were treated with 96 h-SPS or RPMI and normal saline as control, n = 6 for each group. After analysis of iron, lactate, and pH levels in the dissected tumors, Ki67 antigen expression, angiogenesis, and necrosis evaluation were carried out. Gene expression was assessed using RT-qPCR. Moreover, 96 h-SPS composition was discovered by Nano-LC-ESI-MS/MS.

Results: 96 h-SPS solution reduced the LA7 cell viability, proliferation, and migration and Gch1 and Spr genes expression in vitro ($p<0.05$), stemness gene Oct4 was upregulated ($p<0.01$). The intracellular lactate was significantly decreased in the 96 h-SPS treated group ($p=0.007$). In this group, Gch1 and Spr were significantly downregulated ($p<0.05$) and Sox2 and Oct4 expression was not changed significantly. The number of vessels and mitosis (Ki67+ cells) in the 96 h-SPS–treated group was significantly reduced (p 0.024). The increased rate of necrosis in this group was statistically significant ($p=0.04$). Proteomics analysis revealed candidate effectors' components of 96 h-SPS solution.

Conclusion: 96 h-SPS solution may help to prevent cancer stem cell-mediated tumor development. This phenomenon could be mediated through direct cytotoxic effects, inhibition of cell proliferation and migration in association with reduction in Gch1 and Spr genes expression, angiogenesis and mitosis rate, and necrosis augmentation.

Keywords: breast cancer stem cells, LA7, adaptive immunity, serum starvation, nano-LC-ESI-MS/MS, cancer development, immunohistochemistry

Pretreatment of Human Wharton's Jelly-Derived Mesenchymal Stem Cells by Azithromycin Reduces Reactive Oxygen Species

Zahra Sadat Razavi[1], Sara Soudi[1]

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background: Utilizing human Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs), we investigated the in vitro influence of azithromycin (AZM) on the polarization of MSCs.

Methods: WJ-MSCs were cultured in the presence of azithromycin and activated using lipopolysaccharide (LPS). Reactive oxygen species (ROS) detection was performed on these cells via dichlorodihydrofluorescein diacetate (DCF-DA) dye product. We determined the vitality of AZM-treated WJ-MSCs using the MTT test and the annexin V/propidium iodide apoptosis assay to establish the best treatment dose.

Results: AZM-treated WJ-MSCs produced less ROS production.

Conclusion: These findings show that azithromycin influences the inflammatory process at the mesenchymal stem cell (MSC) level and alters MSC polarization toward the alternatively activated phenotype (MSC2).

Keywords: Azithromycin, Mesenchymal stem cell, reactive oxygen species, Polarization, Inflammatory response

Prostaglandin E2 Signaling Through EP2 and EP4 Receptors Reduce Mesocar T Cells Antitumor Function

Behnia Akbari[1], Tahereh Soltantoyeh[1], Zahra Shahosseini[2], Jamshid Hadjati[1], Hamid Reza Mirzaei[1]

1. Tehran University of Medical Sciences, Tehran, Iran.
2. Iran University of Medical Sciences, Tehran, Iran.

Background: The efficacy of Chimeric Antigen Receptor (CAR) T cell therapy has been limited partly due to immunosuppressive tumor microenvironment (TME) in solid tumors. As a soluble component of TME, prostaglandin E2 (PGE2) plays a significant role in the suppression of antitumor immune response. In this study, we are going to not only test mesoCAR T cells efficacy against pancreatic cancer cells but also investigate the impact of pharmacological blockade of PGE2 receptors (EP2/EP4) on mesoCAR T cell function in vitro.

Methods: Using standard molecular methods, second-generation lentiviral transgenes encoding fully human anti-mesothelin CAR (mesoCAR) were produced. To investigate the effect of PGE2 on the function of mesoCAR T cells, these cells were cocultured with tumor cells in the presence and absence of PGE2, and pharmacological inhibitors of the EP2 and EP4 receptors. Then, the cytotoxicity, proliferation, and cytokine production of mesoCAR T cells were measured.

Results: Our findings showed that PGE2 can inhibit the proliferation of T and CAR T cells. Also, this metabolite reduced the antitumor function of mesoCAR T cells. Pharmacological blockade of EP2 and EP4 receptors enhanced the antitumor capacity of mesoCAR T cells to kill pancreatic cancer cells. Following the simultaneous use of EP2 and EP4 antagonists, the inhibitory effect of PGE2 on the antitumor activity of mesoCAR T cells was completely diminished.

Conclusion: The pharmacological targeting of PGE2 inhibitory receptors on the surface of mesoCAR T cells can lead to the resistance of these cells to the inhibitory effects of PGE2 present in the tumor environment. Therefore, it can be hoped that the reduction of EP2/EP4 expression in CAR T cells can be a promising approach for the treatment of solid tumors in clinics in the future.

Keywords: Chimeric Antigen Receptor T cells, Mesothelin, Pancreatic cancer, Pharmacological targeting, PGE2, EP2, EP4.

Role of Nanotechnology in the Improvement of CAR-T Cell Therapy

Kimia Esmaeilzadeh[1]

1. Departments of Medical Nanotechnology School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

Background: One of the most common diseases around the world is cancer. There are various treatment methods such as surgery, chemotherapy, radiation therapy, etc., and one of the new methods of cancer treatment is the use of the Chimeric Antigen Receptor (CAR) T-cell. This type of treatment is based on genetic engineering and immunology. One of the challenges ahead for the use of CAR-T cell therapy is that extracting T cells, expressing CAR on them, and multiplying them in sufficient quantity is a relatively complicated method that requires extensive economic capital. Also, many patients are ineligible for CAR-T cell therapy, as previous treatments and advancing age can compromise the functional status of T cells. Nanotechnology has the potential to address the aforementioned challenges of CAR-T cell therapy.

Methods: We searched Google Scholar, PubMed databases, and Scopus and found 28 articles. 12 articles were selected with specific keywords.

Results: The roles of nanoparticles (NPs) in CAR-T cell therapy: NPs as a carrier for CAR cargo, CAR-T cell monitoring, CAR-T cell expansion, and CAR-T cell modification. Conditions for NPs carrier to carry CAR cargo for manipulation of cells: It must protect cargo from degrading and physiological mechanisms, it should be safe and stable during blood circulation, and should not interact with biomolecules to elicit an immune response, it must be taken up by specific and targeted cells, it must be designed in a way that enhances the transfection efficiency of targeted cells, and upon internalization, it should safely travel along the cell machinery and import its cargo into the cell nucleus.

Conclusion: CAR-T cell therapy represents a special class of immunotherapy based on genetically engineered T cells. Nanoparticles are not a substitute for CAR-T cells, but they improve the efficiency of CAR-T cells to identify tumor markers and identify target cells more easily and destroy them.

Keywords: CAR-T cell therapy, Nanotechnology, Nano-immunotherapeutic, Nano-carriers

T lymphocyte Cytotoxicity against PDL1+ Tumor Cells in The Presence of PDL1-CD3 Bispecific T Cell Engager (Bite) And Adipose-Derived Mesenchymal Stem Cells (ASCs)

Leila Moeinzadeh[1], Amin Ramezani[1], Mahboobeh Razmkhah[1]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Now, cancer immunotherapy (CI) is rapidly advancing and is mostly directed at the immune system or the tumor microenvironment (TME) rather than tumor cells themselves. Due to their ability to recruit immune cells to kill tumor cells directly, Bispecific T-cell engager antibodies (BiTE) hold great potential in T-cell redirecting therapies. However, there are many components in the TME such as mesenchymal stem cells (MSCs) that may interfere with BiTE function. Herein, we designed an anti-PDL1-BiTE that targets PD-L1 and CD3 and investigated its effect on PDL1-positive cancer cells in the presence or absence of adipose derived-MSCs (ASCs).

Methods: Our anti-PDL1-BiTE comprises VL and VH chains of anti-CD3 monoclonal antibody (mAb) linked to the VL and VH chains of anti-PDL1 mAb, that simultaneously bind to the CD3ε subunit on T cells and PD-L1 on tumor cells. Flow cytometry was employed to assess the strength of binding of anti-PDL1-BiTE to tumor cells and T cells. The cytotoxicity of PBLs was evaluated by CFSE assay and flow cytometry after using anti-PDL1-BiTE in the presence or absence of ASCs.

Results: 27.5% (±6.4) of U251-MG tumor cells were killed in the presence of anti-PDL1-BiTE and PBLs, whereas it was 16.1% (±3.5) in PBLs/tumor condition. Anti-PDL1-BiTE in combination with PBLs/ASCs kills 30.6% (±5.8) of PD-L1+ tumor cells. ASCs did not show a significant effect on the biological activity of anti-PDL1-BiTE.

Conclusion: Overall, anti-PDL1-BiTE selectively depletes PD-L1-positive cells and represents a new immunotherapeutic approach. It increases the accumulation of T cells and can improve the prognosis of PD-L1 positive cancers in spite of the immunomodulatory effects of ASCs.

Keywords: Bispecific T-cell engager antibody; PD-L1; Mesenchymal Stem Cells; Immunotherapy

The Anti-Fibrotic Effects of Human Wharton's Jelly Mesenchymal Stem Cells Derived- Exosomes Enriched by Mirna-124 on Murine Model of Liver Fibrosis

Bahare Niknam[1], Davar Amani[1], Kaveh Baghaei[2], Seyed Mahmoud Hashemi[1]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: Liver fibrosis is a major global health challenge that leads to organ failure mediated via the production of inflammatory cytokines and fibrotic biomarkers. Despite advances in the control of liver disease, effective treatment has not yet been provided. Cell therapies-based researchers have identified the anti-inflammatory effects of mesenchymal stem cell-derived exosomes in the healing process of liver fibrosis. Therefore, using the transfer properties of these exosomes, they can be used as effective carriers in anti-inflammatory pathways in the process of liver fibrosis. Given that miR-124 negatively regulates the Signal transducer and activator of transcription 3 (STAT3), and STAT3 is a key regulator in liver fibrosis, we investigated whether enrichment of exosomes by miR-124 can improve the therapeutic efficacy of Human Wharton's jelly mesenchymal stem cells derived exosomes(hWJMSC-Exo) in treating liver fibrosis.

Methods: Exosomes were isolated from Human Wharton's jelly mesenchymal stem cells (hWJMSC) and loaded with miR-124 mimic. An intraperitoneal injection of carbon tetrachloride (CCl4) twice weekly for six weeks induced liver fibrosis. miR-124-enriched exosomes (ExomiR-124) were administered for three weeks in mice and then examined for the relative anti-inflammatory and anti-fibrotic impacts of the ExomiR-124 through histology, biochemistry, Real-time PCR, immunohistochemistry, and ELISA.

Results: In this study, we explored the novel functions of miR-124 in liver fibrosis and showed that upregulation of miR-124 restored the liver function, reduced collagen accumulation, restrained the expression of IL-6, IL-17, TGF-β, STAT3, α-SMA, COL1, and COL3, and thus reduced inflammatory and fibrotic response more effectively than exosomes. These results indicatE improved efficacy upon microRNA enrichment.

Conclusions: ExomiR-124 demonstrated the potential to induce beneficial anti-inflammatory and anti-fibrotic responses. Exosomes, aside from the delivery function of miRNA, revealed anti-inflammatory and anti-fibrotic useful characteristics that could introduce ExomiR-124 as a promising approach in liver fibrosis combination therapies.

Keywords: hWJMSCs, Exosomes, miR-124, Liver fibrosis

The Effect of Exosomes Isolated from Human Wharton's Jelly Mesenchymal Stem Cells Stimulated by Poly (I:C) On CD4+CD25+Foxp3+ Regulatory T Cells

Behjat Misaghian[1], Ata allah Ghadiri[1,2], Ali Asadirad[1], Sareh Amirzadeh[3], Afshin Amari[1,2]

1. Department of Immunology, School of Medicine Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Cellular & Molecular Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3. Department of Infertility, Infertility Research and Treatment Center of ACECR, Ahvaz, Iran.

Background: Mesenchymal stem cells (MSCs) are potential cellular candidates for inflammatory and autoimmune diseases because of their multilineage potential and immunomodulatory and anti-inflammatory function. The limitations of cell therapy have prompted researchers to seek to use cellular products for treatment. Besides the secretion of soluble molecules, the release of exosomes represents an alternative mechanism adopted by MSCs to exert their immunomodulatory and anti-inflammatory effects on target cells through paracrine effects. Inflammatory conditions like Toll-like receptor (TLRs) engagement can change the biological functions of MSCs. TLR3 is highly expressed IN MSCs and their activation can significantly modulate the immunosuppressive and anti-inflammatory functions of MSCs. In this study, we evaluated the effect of exosomes isolated from Poly (I:C) treated human Wharton's jelly mesenchymal stem cells (hWJ-MSCs) on regulatory T Cells.

Methods: We isolated hWJ-MSCs based on explant culture. HWJ-MSCs were treated with Poly (I: C) for 48 hours. Then, exosomes were isolated from the culture supernatants. Exosomes were confirmed by DLS and SEM. Peripheral blood mononuclear cells (PBMCs) isolated from the healthy donors were stimulated with PHA and co-cultured with Poly (I:C) treated hWJ-MSCs derived exosome and untreated hWJ-MSCs derived exosome or without hWJ-MSCs-derived exosome for 6 days. Then, the frequency of CD4+CD25+ Foxp3+ regulatory T cells was measured by flow cytometry.

Results: Our results showed that exosomes isolated from Poly (I:C) treated hWJ-MSCs significantly increased the frequency of CD4+CD25+ Foxp3+ regulatory T cells compared to the untreated hWJ-MSCs derived exosome group ($p<0.05$) and control group ($p<0.05$).

Conclusion: Stimulation of TLR3 can improve the anti-inflammatory properties of exosomes derived from hWJ-MSCs via an increase in the frequency of Treg cells.

Keywords: Exosome, TLR3, Mesenchymal Stem Cells, Regulatory T cells

The Effect of Mesenchymal Stem Cell-Derived Supernatant Nasal Administration on Lung Inflammation and Immune Response in BCG-Vaccinated BALB/C Mice

Ali Hazrati[1], Abolfazl Chenari[2], Ahmad Zavaran Hosseini[2], Mahdieh Motiee[2], Sara Soudi[2]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background: Mesenchymal stem cells (MSCs) are among the known cells that can control and modulate immune responses in different circumstances, including autoimmune diseases. Also, various studies have shown that they can prevent and reduces the pulmonary inflammation caused by infectious agents. In the case of tuberculosis and inflammation caused by BCG, the granuloma has destructive effects and improper orientation of the immune response. Therefore, it is possible to prevent airway damage by preventing harmful inflammatory responses and guiding the immune system responses. This study investigates the role of nasal administration of MSCs supernatant by designing an inflammatory model in the BALB/c mice lung with BCG.

Methods: MSCs are isolated from mice adipose tissue in this study and evaluated for their phenotypic and differentiation properties. After the third passage, these cells' condition medium (CM) was collected. 20 mice were divided into four groups. Group 1 receive BCG (107 CFU in 5 ml volume for 15 min) nasal administration. Group 2 treated with CM, and group 3 initially were treated with CM (in 5 ml volume for 15 min) and, after 24 hr, treated with BCG nasal administration. CM treatment was continued every five days for one month. The fourth group of mice was treated with PBS nasal administration of CM and BCG. One week after the last administration, the lung tissue of mice in each group was pathologically examined. In addition, secretion of IL1-β, IL-6, TNF-α, TGF-β, and IL-10 in the alveolar fluid and secretion of IL-4 and IFN-γ cytokines in the supernatant of splenocytes was evaluated by ELISA.

Results: The level of expression and production of inflammatory cytokines in the CM-treated group was associated with a significant decrease, and the pathology of the lung tissue showed improvement. Also, the TNF-α / IL-10 ratio in the alveolar lung fluid of the BCG-received group is 2/9 and decreased to 0.58 after successive CM treatment.

Conclusion: Therefore, it can be concluded that inflammatory responses to BCG infection in the presence of CM are balanced and pave the way for the induction of effective immune responses by reducing lung tissue damage than each alone.

Keywords: Mesenchymal Stem Cell, Supernatant, BCG, Nasal Administration, Lung, Inflammation

The Effects of Dexamethasone-Loaded Exosomes on Lung Injury in CLP-Induced Sepsis Mice

Zahra Mirsanei[1], Fatemeh Ahangari[1], Sara Soufi[2], Fatemeh Jamshidi[3], Solaiman Ali Alhashmi[3], Saeed Vakilian[3], Sara Soudi[4], Seyed Mahmoud Hashemi[1]

1. Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran.
2. University of Tabriz of Medical Sciences (SBMU), Tabriz, Iran.
3. University of Nizwa
4. Tarbiat Modares University, Tehran, Iran.

Background: The regenerative effect of umbilical cord mesenchymal stem cells-derived exosomes in tissue healing, along with the anti-inflammatory effect of dexamethasone on inflammatory diseases such as COVID-19, promoted us to examine the efficacy of dexamethasone-loaded exosomes on lung damage caused by sepsis

Methods: Cecal ligation and puncture (CLP) model was used to induce sepsis in mice. C57BL/6 mice injected with sham, CLP-induced sepsis, CLP-induced sepsis mice injected with exosomes, CLP-induced sepsis mice injected with dexamethasone, and CLP-induced sepsis mice injected with dexamethasone-loaded exosomes. The mice were sacrificed after 24 hours, tissue samples were collected for H&E staining, and BAL fluid was collected to examine protein and leukocyte changes.

Results: According to the results of a study conducted on sepsis mice, in comparison to the CLP group, the number of infiltrated immune cells, congestion, and alveolar wall thickness in lung tissue, and the total protein and leukocyte population in BAL fluid were significantly reduced in the dexamethasone-loaded exosomes treated group.

Conclusion: As a nanoparticle carrying very small amounts of dexamethasone compared to the administration of dexamethasone alone, exosomes loaded with dexamethasone can effectively reduce pulmonary inflammation in sepsis patients and reducE systemic dexamethasone side effects.

Keywords: Cecal Ligation and Puncture (CLP), exosomes, dexamethasone, Lung injury

The Inhibitory Effects of Exosomes Derived from Oncolytic Reovirus-Infected Adipose Mesenchymal Stem Cells with Therapeutic Potency on Mice Model of Colorectal Tumor

Ala Habibian[1], Hoorieh Soleimanjahi[1], Seyed Mahmoud Hashemi[2], Seyed-Mahmood Seyed-Khorrami[1]

1. Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Over the last few years, virotherapy with oncolytic viruses attract more attention. Using mesenchymal stem cells and their secretomes, especially exosomes in combination with oncolytic viruses may be a helpful approach against cancer. In this study, we employed a novel strategy combining therapeutic agents, including mesenchymal stem cells-derived exosomes, with oncolytic reovirus.

Methods: Exosomes were isolated from mouse adipose tissue. Moreover, infected exosomes with oncolytic reovirus were purified from infected mesenchymal stem cells. The appropriate concentration of the uninfected and infected exosomes was determined for apoptosis on the base of in vitro phase. The BALB/c mice received CT26 colorectal tumors and were treated with intra-tumoral administration of exosome, exosome, and reovirus oncolytic simultaneously, infected exosome with oncolytic reovirus, oncolytic reovirus, and PBS. Finally, the size of tumors, immune response indicators, and cytotoxicity were evaluated.

Results: The current study revealed that the infected exosomes with oncolytic reovirus in 200 µg/ml concentration performed the most apoptosis of tumor cells in 48 hours in vitro. Tumor recession in the infected exosome with the reovirus group increased slightly compared with the control group. On the other hand, investigation of stimulated splenocytes revealed that the most efficient therapy was determined in infected exosomes with the oncolytic reovirus group through cytotoxicity assay. Furthermore, IFN-γ and TGF-β secretion in treated groups related to exosome and oncolytic reovirus were significantly increased in comparison with the control group.

Conclusion: Using the therapeutical platform of oncolytic reovirus which is surrounded by mesenchymal stem cells-derived exosomes can play an effective role in the inhibition of colorectal tumor growth and proliferation and could apply as a cell-free therapeutical candidate for colorectal cancer therapy.

Keywords: Ras signaling pathway, Oncolytic reovirus, Nanovesicle, Colorectal cancer

The Protective Efficacy of Human Wharton's Jelly Mesenchymal Stem Cells Secretomes to Induce Death in Breast Cancer Cell Line SKBR3

Samaneh Karimi[1]

1. Abadan University of medical sciences, Abadan, Iran.

Background: Breast Cancer (BC) is caused by the abnormal growth of cells in the breast. This study's main goal was to assess the protective effects of human Wharton's jelly mesenchymal stem cell secretomes (hWJMSCs-Se) on the growth and progress of the BC SKBR3 cell line in addition to the changes in apoptosis genes following treatment.

Methods: SKBR3 cells have treatment with 10, 25, and 50 µg/mL hWJMSCs-Se for 24 and 48 hours. The SKBR3 cells' cytotoxicity and proliferative potential were assessed using the MTT test and colony formation. Apoptosis was estimated using cell cycle arrest and annexin V/PI staining. Important apoptosis-related genes' mRNA expression levels were also looked at.

Result: Colony counts and viability percentages in the hWJMSCs-Se-treated SKBR3 cells significantly declined as a function of concentration and time. In comparison to the control, the hWJMSCs-Se-treated SKBR3 cells had a significantly higher apoptotic index. According to FACS analysis, hWJMSCs-Se inhibited cell cycle development at G0/G1 and blocked proliferation in a dosage-dependent manner. The hWJMSCs-Se-exposed SKBR3 cells had significantly higher Caspase-9 and -3 activities. The hWJMSCs-Se-treated SKBR3 cells exhibited significantly augmented expression of caspase-9, caspase-3, and the Bax/Bcl-2 proportion. The hWJMSCs-Se didn't have an impact on caspase-8 activity.

Conclusion: Our investigation showed, the hWJMSCs-se antagonist offers a novel approach to anticancer therapies for the treatment of HER2-positive malignancies. It significantly reduced BC cell growth and increased cell death, primarily through the intrinsic apoptotic pathway, depending on time and concentration.

Keywords: Mesenchymal stem cell, Apoptosis, Secretome, Breast Cancer, Proliferation

Therapeutic Effects of Azithromycin (AZT) and Extracellular Vesicles Derived from Mesenchymal Stem Cells (MSC-Evs) Injection on the Cecal Ligation and Puncture (CLP) Model of Sepsis

Fatemeh Ahangari[1], Zahra Mirsanei[21], Sara Soufi[2], Sara Soudi[3], Sahar Ghaffari Khaligh[4], Seyed Mahmoud Hashemi[1]

1. *Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran.*
2. *Tabriz University of of Medical Sciences (SBMU), Tabriz, Iran.*
3. *Tarbiat Modarres University (TMU), Tehran, Iran.*
4. *Semnan University of Medical Sciences, Semnan, Iran*

Background: Recent studies showed that the administration of azithromycin (AZT) and extracellular vesicles derived from mesenchymal stem cells (MSC-EVs) can defend body organs from the destructive effects of sepsis in mouse models by means of reducing inflammation, increasing immunomodulation, and the induction of M2 macrophages.

Methods: Mice were randomly placed into five different groups, including the sham group, the cecal ligation and puncture (CLP) group, the AZT group, the MSC-EVs group, and the AZT+MSC-EVs group. The CLP group received sterile saline, the AZT group received 100 mg/kg of AZT, the MSC-EVs group received 200 mg/kg of MSC-EVs, and the AZT+MSC-EVs group received 100 mg/kg of AZT and 200 mg/kg of MSC-EVs. After 24 hours mice were sacrificed. The serum was separated from blood clots and biochemical parameters, including ALT, AST, BUN, and creatinine were measured. Moreover, the lung, liver, and kidney of each mouse were placed in tubes containing 10% formalin, and, eventually, pathological slides were prepared from them. The peritoneal lavage of mice was cultured on trypticase soy agar (TSA) and the colony-forming unit of bacteria was counted after 24 h. The number of neutrophils and lymphocytes was counted using a microscope.

Results: AST and creatinine levels of serum reduced significantly ($p<0.05$) in the AZT+MSC-EVs group in comparison with the CLP group. However, ALT and BUN levels in the serum of mice receiving combinational therapy of AZT+MSC-EVs did not decrease significantly. None of the treatment groups could reduce ALT levels significantly. However, each single treatment group, including the AZT group and the MSC-EVs group could decrease the BUN levels in comparison with the CLP group. In addition, this combinational therapy can significantly reduce the histopathological scores of kidneys in comparison with the AZT group ($p<0.05$). Also, the histopathological scores of the liver reduced significantly in the combinational therapy group rather than in other groups ($p<0.05$). The bacteria count was reduced significantly in all of our treatment groups ($p<0.05$). The neutrophile to lymphocyte ratio was decreased significantly in merely MSC-EVs groups ($p<0.05$).

Conclusion: The co-administration of AZT and MSC-EVs with the dose of 100 mg/kg and 200 mg/kg, respectively, could reduce biochemical levels in the serum, including AST and creatinine. The histopathological scores showed that the treatment with MSC-EVs+AZT can reduce injury scores. Moreover, the neutrophil to lymphocyte ratio which is an indicator of inflammation, and bacteria count which is an indicator of infection dampened in our combinational therapy group. This study showed the promising therapeutic potential of MSC-EVs+AZT in comparison with either of the treatment groups individually.

Keywords: Extracellular Vesicles, Exosomes, Mesenchymal stem cells, Sepsis

Therapeutic Effects of Azithromycin (AZT) and Extracellular Vesicles Derived from Mesenchymal Stem Cells (MSC-Evs) Injection on Tissue Damage in the Cecal Ligation and Puncture (CLP) Model of Sepsis

Fatemeh Ahangari[1], Zahra Mirsanei[1], Sara Soufi[2], Sara Soudi[3], Sahar Ghaffari Khaligh[4], Seyed Mahmoud Hashemi

1. *Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran.*
2. *Tabriz University of of Medical Sciences (SBMU), Tabriz, Iran.*
3. *Tarbiat Modarres University (TMU), Tehran, Iran.*
4. *Semnan University of Medical Sciences, Semnan, Iran*

Background: Recent studies showed that the administration of azithromycin (AZT) and extracellular vesicles derived from mesenchymal stem cells (MSC-EVs) can defend body organs from the destructive effects of sepsis in mouse models. The hypothesis of this study was based on the evaluation of a combinational therapy consisting of AZT and MSC-EVs on biochemical parameters, including alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine, and pathological scores.

Methods: C57BL/6 female mice were randomly divided into the sham group, the cecal ligation and puncture (CLP) group, the AZT group, the MSC-EVs group, and the AZT+MSC-EVs group. The CLP group underwent abdominal surgery and received sterile saline via intravenous injection (i.v.), the AZT group received 100 mg/kg AZT via intraperitoneal injection (i.p.), the MSC-EVs group received 200 mg/kg of MSC-EVs via i.v. injection and the AZT+MSC-EVs group received an i.p. injection of 100 mg/kg of AZT and a tail vein injection of 200 mg/kg of MSC-EVs. After 24 hours mice were euthanized by exsanguination under anesthesia. The serum was separated from blood clots and biochemical parameters, including ALT, AST, BUN, and creatinine were measured. Moreover, the lung, liver, and kidney of each mouse were placed in tubes containing 10% formalin, and, eventually, pathological slides were prepared from them.

Results: AST and creatinine levels of serum reduced significantly (p<0.05) in the AZT+MSC-EVs group in comparison with the CLP group. However, ALT and BUN levels in the serum of mice receiving combinational therapy of AZT+MSC-EVs did not decrease significantly. None of the treatment groups could reduce ALT levels significantly. In the AZT+MSC-EVs group, the level of BUN was not significantly reduced compared to the CLP group. However, each single treatment group, including the AZT group and the MSC-EVs group could decrease the BUN levels in comparison with the CLP group. In addition, this combinational therapy can reduce pathological scores of kidneys significantly in comparison with the AZT group (p<0.05). Also, pathological scores of the liver reduced significantly in the combinational therapy group rather than in other groups (p<0.05). However, the pathological scores of lungs reduced significantly in the merely MSC-EVs group (p<0.05).

Conclusion: The co-administration of AZT and MSC-EVs with the dose of 100 mg/kg and 200 mg/kg, respectively, could reduce some biochemical levels in the serum, including AST and creatinine. However, the pathological scores showed that MSC-EVs+AZT can reduce injury scores, and despite lungs, it can effectively protect the liver and kidneys.

Keywords: Extracellular Vesicles, Exosomes, Mesenchymal stem cells, Sepsis

Therapeutic Potential of Mesenchymal Stem Cells Expressing IDO1 Transgene in Modulating Inflammation in Surgical-Induced Experimental Spinal Cord Injury

Nayere Parhizkar[1], Ahmad reza Bahrami[2], Azadeh Haghighitalab[2], Soodeh Alidadi[1], Hossein Kazemi mehrjerdi[1]

1. Department of veterinary medicine Faculty of Ferdowsi University of Mashhad
2. Department of Science Faculty of Ferdowsi University of Mashhad

Background: With few therapeutic options and devastating effects, improving the functional outcome of a spinal cord injury (SCI) is a primary objective. Neuroinflammation, which may be a target for better recovery and quality of life following damage, contributes to the outcome of SCI. It is known that mesenchymal stem cells (MSCs) may be used to treat CNS damage. The immunomodulatory activity of MSCs is carried out by changing pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages. In addition, MSCs encourage a reduction in inflammation by secreting cytokines and immunomodulatory substances, including indoleamine 2,3-dioxygenase (IDO). Here, we employed MSCs overexpressing IDO1 to treat a rat model of compression SCI.

Methods: 30 adult female rats were randomly divided into 3 equal groups: IDO1, sham, and SCI groups. Under anesthesia, the sham group only underwent laminectomy surgery, and in the experimental and SCI groups, compression injury of the spinal cord was created using a vascular clip at the T9–10 level of the spinal cord. Then the hydrogel containing the cells (1 × 106) was in sight at the lesion site. The histology of the injury site and WBC changes in all 3 groups were evaluated after 42 days.

Results: The lower inflammatory responses in the IDO1 group when compared to the SCI and sham group were confirmed by histopathological analysis of the lesion sites. The reduction in inflammatory cell counts following cell application to the lesion site and the histological findings support the immunomodulatory actions of IDO1.

Conclusion: These results suggest that MSCs that overexpress IDO1 may contribute to the healing process of spinal cord tissue after SCI through an immunomodulatory mechanism.

Keywords: mesenchymal stem cells (MSCs), immunomodulation, indoleamine 2, 3-dioxygenase (IDO), Spinal cord injury (SCI)

Tideglusib Enhances the Expression of Several Immunomodulatory Factors in Bone Marrow-Derived Mesenchymal Stem Cells

Halimeh Hassanzadeh[1], Ahmad Reza Bahrami[1,2], Mahboubeh Kazemi Noughabi[3], Hamid Reza Bidkhori[4], Maryam M. Matin[1,2]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
4. Stem Cells and Regenerative Medicine Research Group, Academic Center for Education, Culture, and Research (ACECR)-Khorasan Razavi, Mashhad, Iran

Background: It is well known that mesenchymal stem cells (MSCs) are among the most promising stem cells for the treatment of several inflammatory and immune diseases due to their potent immunoregulatory properties. Tideglusib is available on the market as an inhibitor of glycogen synthase kinase-3, a protein that plays an important role in the immune response. The aim of this study was to investigate the effects of tideglusib on the expression of a number of immunomodulatory genes in human bone marrow (BM)-derived MSCs.

Methods: BM-MSCs were derived from patients and characterized by immunophenotyping, as well as assessment of their adipogenic and osteogenic differentiation potential. In the next step, the BM-MSCs were treated with 200 nM tideglusib for 24 h. The expression of target genes was then verified in preconditioned cells as well as the BM-MSCs without any treatment via quantitative RT-PCR.

Results: The expression of immune modulatory genes, such as TDO-2, COX-2, and TSG-6 was increased ($p<0.01$) in BM-MSCs after tideglusib treatment. Comparing the test group with the control group, we found a decrease ($p<0.01$) in the expression of IL-6 as a pro-inflammatory cytokine in the test group.

Conclusion: It is suggested that tideglusib plays a significant role in the upregulation of immunomodulatory genes in human BM-MSCs, and has the potential to manipulate the phenotype of MSCs to anti-inflammatory in a way that inhibits the production of pro-inflammatory cytokines such as IL-6. Hence, it may be introduced as a new class of small molecules that promote the immunomodulatory properties of MSCs and therefore can improve their clinical applications.

Keywords: "Mesenchymal Stem Cells", "Tideglusib", "Immunomodulation", "Gene Expression"

Using A Non-Viral Gene Transfer Method for More Efficient Co-Culture and Proliferation of Hematopoietic Stem Cells and with An Approach to Stem Cell Therapy

Omid Moeini[1], Monireh Ajami[2], Amir Atashi[3], Masoud Soleimani[4], Maedeh Mashhadi ali tehrani[5], Mansoureh Ajami[3]

1. Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
2. Department of Hematology, Faculty of Paramedical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
3. Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Shahroud University of Medical Sciences, Shahroud, Iran
4. Department of Hematology and Blood Banking, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
5. Student Research Committee, School of Paramedicine, Shahroud University of Medical Sciences, Shahroud, Iran

Background: Hematopoietic stem cells (HSCs) are an attractive source for cancer and leukemia immunotherapy also cord blood CD34+ cells are advantageous for use as an immunotherapy. On the other hand, the big problem is that these cells are limited and the amount of CD34+ HSC is insufficient. In the present study, to obtain an efficient proliferation of hematopoietic stem cells, umbilical cord blood mesenchymal stem cells with increased expression of sSCF, mSCF, and SDF-1 were used in coculture with cord blood CD34+ hematopoietic cells.

Methods: MSCs and CD34+ HSCs were isolated from UCB mononuclear cells. UCB mesenchymal stem cells were nucleofected with one or more expression vectors of sSCF, mSCF, and SDF-1 Then, isolated CD34 + HSC cells were cultured with nuclear MSCs in 10 groups in a culture medium containing TPO and Flt3L with or without SCF (groups 3F or 2F. After that, the number of CD34 + HSCs, clonogenic capacity, and the transcription levels of known regulatory genes and stemness of HSCs (CXCR4, HOXB4, BMI1, and SALL4) were evaluated in co-culture with modified MSCs.

Results: CD34 + HSCs which expanded on MSCs overexpressing mSCF/sSCF/ SDF-1 in the 3F group showed the most significant increase in the expansion (4.73 ± 0.26 fold), clonogenic capacity (5.3 ± 0.25 fold) and also transcriptional levels of CXCR4, HOXB4, and BMI1 (3.49 ± 0.13, 9.49 ± 0.78, and 11.6 ± 0.9 fold), respectively ($p<0.05$).

Conclusion: The results of the present study indicate the fact that the presence of a suitable concentration of SDF-1 along with a certain amount of SCF and FLT3 cytokines can have an impressive effect on the proliferation of hematopoietic umbilical cord blood primary cells.

Keywords: Stem cell therapy, Hematopoietic Stem Cell, Mesenchymal Stem Cell, Co-culture, Umbilical Cord Blood, Stromal Cell-Derived Factor 1, Stem Cell Factor

Uterine Adipose Tissue-Derived Mesenchymal Stem Cells from Pregnant and Non-Pregnant Mice Have Distinct Immunomodulatory Properties

Fatemeh Rezaei Kahmini[1], Shahab shahgaldi[2], Maryam Azimi[3]

1. Autoimmune Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3. Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran

Background: The immunomodulatory properties of mesenchymal stem cells (MSCs) have been well documented. However, resident MSCs in various tissue might have distinct immunomodulatory properties which indicate different therapeutic potential. The microenvironment in the pregnant uterus is substantially different from the non-pregnant uterus. So, the aim of this study was to compare the immunomodulatory properties of MSCs isolated from uterine adipose tissue from pregnant and non-pregnant mice.

Methods: Uterine adipose tissue-derived MSCs isolated from pregnant or non-pregnant BALB/c mice and characterized by immunophenotyping and differentiation potential to mesodermal lineage. The immunomodulatory properties of MSCs-conditioned media (CM) were assessed through co-culture of either pregnant or non-pregnant MSCs-CM with splenocytes. Following 72 hours of co-culture, cytokines production (IL-4, IL-10, TGF-β, and IFN-γ) by splenocytes were determined using ELISA.

Results: MSCs isolated from pregnant mice (P-MSCs) secreted higher levels of TGF-β and IL-10 compared to non-pregnant mice (N-MSCs). Of note, P-MSCs or N-MSCs secreted comparable amounts of IL-4 and IFN-γ. The immunomodulatory effect of MSCs-CM on splenocytes showed that P-MSCs induce the production of anti-inflammatory cytokines (IL-4, IL-0, and TGF-β) by splenocytes more efficiently compared to N-MSCs.

Conclusion: Resident uterine MSCs showed immunomodulatory properties, however, it seems that P-MSCs have a distinct immunomodulatory function which indicates its therapeutic potential and candidate them as a new target of immunotherapy to modulate the immune system.

Keywords: Mesenchymal stem cell, Immunomodulation, Cell therapy, Pregnancy

Vitamin E and Selenium Improve Mesenchymal Stem Cell Conditioned Media Immunomodulatory Effects

Fereshteh Ghasemi[1], Majid Khoshmirsafa[1], Elahe Safari[1], Samane Khorrami[1]

1. Department of Medical Immunology, School of Medicine, Iran University of Medical Science, Tehran, Iran

Background: Mesenchymal stem cells (MSCs) with immunoregulatory properties affect immune systems. Many studies showed that antioxidants such as vitamin E (Vit E) and selenium (Se) could improve stem cells survival. This study aims to investigate the effects of MSC-conditioned media (CM) treated with Vit E and Se on dendritic cells (DCs).

Methods: MSCs were isolated from adipose tissue and cultured with Vit E and Se. Immature DCs were cultured with MSC CM treated with Vit E and Se. The expression of HLA-DR, CD86, CD40, and CD83 on mature DC was evaluated. DC supernatant was collected for the study of TGF-β, IL-10, and IL-12.

Results: MSC CM increased CD40 on myeloid DC (mDC). CD40 has been decreased in DC treated with MSC (Vit E) and MSC (Se) CM. HLA-DR expression on DCs and IL-12 levels were significantly reduced in MSC (Vit E) CM. IL-10 concentration increased in DCs treated with MSC (Vit E) and MSC (Se) CM.

Conclusion: According to the results, the treatment of MSC with Vit E and Se enhanced the ability of MSCs to inhibit DCs and improved immunomodulatory effects.

Keywords: Mesenchymal stem cell (MSC), dendritic cell (DC), selenium (Se), vitamin E (Vit E)

Wharton's Jelly Mesenchymal Stem Cells-Derived Exosomes and Imipenem in Combination Reduces Apoptosis and Inflammatory Responses in E. Coli-Infected Hepg2 Cells

Ali Hazrati[1], Sara Soudi[2]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Background: Antibiotics are used to treat bacterial liver infections and the resulting inflammation. However, their use is limited due to their side effects, especially the development of antibiotic resistance. Mesenchymal stem cells (MSCs) are recognized for their immunomodulatory properties. In this study, we investigated the immunomodulatory effect of Wharton's jelly MSC-derived exosomes in combination with imipenem on HepG2 cells infected with Escherichia coli.

Materials and Methods: MSC-derived exosomes were separated from MSCs, which were themselves isolated by flow cytometry. Scanning electron microscopy and dynamic light scanning were used to confirm the presence of exosomes. Quantitative real-time PCR, ELISA, and nitric oxide assay were used to assess the inflammatory response in the infected cells. Annexin-PI was used to measure the extent of apoptosis.

Results: The results showed that the combination of imipenem and MSC-derived exosomes were more effective than imipenem or exosomes alone in reducing the production and secretion of inflammatory cytokines, nitric oxide, and apoptotic rate in E coli-infected HepG2 cells.

Conclusion: According to the results of this study, it can be said that the combined treatment of imipenem and MSC-EXO by involving different pathways can be a better treatment than each alone.

Keywords: Mesenchymal stem cells, Exosomes, Escherichia coli, Inflammation; Immunomodulation

Tolerance and Autoimmunity

Effects of Gliadin on Autoimmune Responses of Central Nervous System of C57BL/6 Mice

Parisa Esmaeili[1,2], Elham Rostami[3], Arefeh Akbarijavar[4], Alireza Akbari Meyestani[5], Hamed Bashiri[6], Mohammad Ali Rezaee[7], Shohreh Fakhari[1,9]

1. Department of Immunology and Hematology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Department of Epidemiology, Pasteur Institute of Iran, Tehran, Iran
3. Department of Biology, School of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
4. Graduated from the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
5. National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
6. Department of Laboratory Sciences, Faculty of Paramedical, Kurdistan University of Medical Sciences, Sanandaj, Iran
7. Zoonoses Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
8. Department of Medical Laboratory Sciences, Faculty of Paramedical, Kurdistan University of Medical Sciences, Sanandaj, Iran
9. Cancer and Immunology Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran

Background: Gluten sensitivity contributes to various degrees of neurological manifestations and neurodegenerative immunological changes. We investigated the experimental features of anti-gliadin immune responses in the central nervous system (CNS) of mice.

Methods: Female C57BL6 mice were divided into three groups. Mice were immunized with complete Freund's adjuvant (CFA) or gliadin emulsified in CFA, and the control group received phosphate-buffered saline (PBS). Immunohistochemistry, hematoxylin-eosin, and Luxol fast blue staining were performed on the sections. The serum levels of interleukin (IL)-17 and interferon-gamma (IFN-γ) were measured using enzyme-linked immunosorbent assay (ELISA). Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to assess the mRNA levels of chemokine (C-X-C motif) ligand-2 (CXCL-2), C-C motif chemokine ligand-2 (CCL-2), and CXCL-10.

Results: In gliadin+CFA immunized mice, the microscopic lesions included perivascular edema, focal-microgliosis, and acute neuronal necrosis in the cortex, subcortical, Purkinje cell layer, and ventral horn of the spinal cord. While extravasation of anti-IgG antibodies and selective targeting of Purkinje cells were observed in gliadin+CFA immunized mice. A significant increase in serum IL-17 and IFN-γ levels ($p<0.05$), as well as expression of CXCL-2, CCL-2, and CXCL-10 in mice immunized with gliadin+CFA, were monitored versus controls.

Conclusion: Our findings indicated that the immune responses directed against gliadin peptides might contribute to blood-brain barrier breakdown, extravasation of serum anti-IgG, gliosis, and acute neuronal necrosis in the cortex and cerebellar Purkinje cells. Anti-IgG antibodies may cause extravasation of blood-born anti-gliadin antibodies and selective targeting of Purkinje cells observed in mice immunized with peptide tryptic (pt) -gliadin in CFA.

Keywords: Central nervous system, Gliadin; Immunity, Neurological disorder

Effect of IFN-B Treatment on Microglia in a Patient with Multiple Sclerosis

Ladan Bajelan[1], Mobin Mohammadi[2], Dariush HaghMorad[3], Nadia Sharafkandi[4], Mohammad Reza Rahmani[4], Abbas Ali Amini[4]

1. Cellular and Molecular Research Center, Research Institute for health development, Kurdistan University of Medical Sciences, Sanandaj– Iran
2. Cancer and Immunology Research Center, Research Institute for health development, Kurdistan University of Medical Sciences, Sanandaj– Iran
3. Department of Pathology and Laboratory Medicine, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
4. Department of Immunology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj– Iran

Background: Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system (CNS). Interferon-beta (IFN-β) is a widely used therapeutic immunomodulatory agent in treating MS. Microglia are the resident immune cells of the CNS that play an important role in CNS homeostasis. In MS patients, the destruction of myelin in the CNS is associated with activated macrophage or microglia, which are thought to be involved in the disease pathogenesis. By considering the importance of microglial cells in the immunopathogenesis of MS, in this study, we investigate the effects of IFN-β on microglial-related genes in the brain of experimental autoimmune encephalomyelitis (EAE) induced mice.

Methods: C57BL/6 mice in the age range of 6-8 weeks were applied and EAE induced by myelin oligodendrocyte glycoprotein (MOG) peptide + complete Freund's adjuvant and treated with Bordetella pertussis toxin. Mice were divided into three groups: EAE induced group treated with 10000 IU s.c IFN-β every other day; the patient group that EAE induced but just received PBS and finally the healthy control group. After isolating the whole brain mRNA extract and cDNA synthesis, we evaluated the expression of TREM-2, IGF-1, and STAT6.

Results: according to the results gene expression rate in the treated group was not significant except in STAT6 and it was higher than in the untreated patient group. For example, gene expression of STAT6 in the healthy control group was 0.02922 which was slightly higher than the expression rate in the IFN-β treated patient group (0.02668). Also, the expression of this gene in the treated group was significantly (p=0.001) higher than in the patient group (p<0.0001). (Numbers represent the average between groups).

Conclusion: IFN-β was able to control the activation of microglia in the brain and shifted them to M2 anti-inflammatory state.

Keywords: EAE, MS, IFN-β, TREM-2, STAT6

Alternative C5 - Convertases Promote Skin Inflammation in Experimental Epidermolysis Bullosa Acquisita

Sadegh Mousavi[1], Shahzad Famil.Dabiri[2], Mayumi Kamaguchi[1], Kentaro Izumi[1], Sören Dräger[3], Leon Schmidt-Jiménez[1], Anika Kasprick[1], Detlef Zillikens[3], Sabrina Patzelt[1], Shirin Emtenani[1], Manuela Pigors[1], Katja Bieber[1], Enno Schmidt[1], Ralf Ludwig[1]

1. Lübeck Institute of Experimental Dermatology, University of Lübeck
2. Islamic Azad University, Pharmaceutical Sciences Branch, Tehran, Iran
3. Department of Dermatology, Allergology and Venereology, University of Lübeck, Lübeck, Germany

Background: Pemphigoid diseases (PDs) comprise a group of blistering skin conditions in which autoantibodies against basal keratinocyte antigens cause loss of cell adhesion to the dermal-epidermal junction (DEJ).

Methods: The deposition of linear IgG and C3 deposits are diagnostic hallmarks of PD. Blockade of the C5/C5aR1 axis has previously been identified as a key driver of skin inflammation in experimental murine PD. However, the functional importance of C3 in PD has not been conclusively addressed. We here, using antibody transfer-induced PD models in C3-deficient mice, demonstrate that in three pre-clinical PD models, namely epidermolysis bullosa acquisita (EBA), bullous pemphigoid (BP), and mucous membrane pemphigoid (MMP), experimental PD develops independently of C3. This indicates that C5a in these model conditions can be proteolytically generated independently of C3. Thus, we next addressed the contribution of alternative C5-convertases using the antibody transfer-induced EBA mouse model.

Results: EBA was induced in neutrophil-elastase (NE) deficient mice and in (WT) mice treated with the thrombin inhibitor argatroban, both considered alternative C5 convertases. While in NE-deficient mice, EBA developed like in wild-type littermate controls, blockade of thrombin led to a significant reduction of clinical disease severity in antibody transfer-induced EBA. However, the degree of reduction in clinical disease activity was not as pronounced when compared to mice deficient in the C5/C5aR1-axis. Thus, additional alternative pathways for C5a generation are most likely operative. Experiments addressing the contribution of elastase and thrombin in established MMP and BP mouse models are ongoing.

Conclusion: Preliminary data suggest that thrombin is also required for MMP induction. In summary, we here provide evidence that skin inflammation in experimental PD can develop independently of C3 and that C5a is, at least partially, generated by thrombin.

Keywords: Complement, Pemphigoid, inflammation, C5 convertase

An Interleukin 12 B Single Nucleotide Polymorphism Increases IL-12p40 Production and Is Associated with Increased Disease Susceptibility in Patients with Relapsing-Remitting Multiple Sclerosis

Mohammad Reza javan[1]

1. Zabol University of Medical Sciences, Zabol, Iran

Background: Through mounting genetic investigations, it has been established that IL12B and IL23R gene single nucleotide polymorphisms have significant associations with autoimmune diseases including inflammatory bowel disease, psoriasis, and ankylosing spondylitis. The IL-12/IL-23 pathway plays a pivotal role in the etiopathogenesis of multiple sclerosis (MS), as suggested by studies both in patients and animal models.

Methods: In a case-control study, 145 MS patients and 200 healthy subjects were genotyped for polymorphisms in IL12B and IL23R genes using Real-Time PCR allelic discrimination approach. Additionally, quantitative analysis of mRNA expression of IL12B in Peripheral Blood Mononuclear Cells from patients and controls was conducted through Real-Time PCR using the TaqMan Gene Expression Assay.

Results: The rs6887695 single-nucleotide polymorphism (SNP) in the IL12B gene showed an association with susceptibility to MS. GG genotype of this variation was more frequent in patients. mRNA expression of IL12B was upregulated in patients. Expression of IL12B mRNA in both MS patients collectively and those with GG genotype for rs6887695 SNP correlated negatively with the onset age of MS patients.

Conclusions: The GG genotype of rs6887695 SNP in the IL12B gene plays a role in the etiopathogenesis of MS.

Keywords: IL12B, IL23R, Multiple sclerosis, SNP, gene expression

Analysis of PD-1 Expression on NK Cells of Patients with Rheumatoid Arthritis; Positive Association with DAS28

Maryam Hemmatzadeh[1], Alireza Ghanavatinejad[2], Farhad Jadidi-Niaragh[1], Hamed Mohammadi[3]

1. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran

Background: Natural killer (NK) cells have been reported to play a critical role in rheumatoid arthritis (RA) pathogenesis. Programmed cell death-1 (PD-1) is a key immune checkpoint receptor expressed by NK cells. Here in this investigation, we determined PD-1 expression on NK cells in RA Patients.

Methods: Peripheral blood specimens were obtained from 45 RA patients and 15 healthy controls. Based on the DAS28 score, the patients were divided into low, moderate, and high-activity groups. PBMCs were isolated from patients with RA and healthy controls using Ficoll-Hypaque. The frequency of PD-1–expressing NK cells was analyzed by flow cytometry.

Results: The percentage of the PD-1–expressing NK cells and Mean fluorescence intensity (MFI) of PD-1 expression on the NK cells were significantly higher in the RA cases compared to the controls (p=0.007 and p=0.003, respectively). PD-1 expression on the NK cells in RA patients had a positive correlation with DAS-28 (r=0.39, p=0.008).

Conclusion: The results of the present study showed that the PD-1+NK cells increased with the positive correlation of disease severity in RA patients. This issue may provide new insights to better comprehend the role of NK cells in the pathophysiology of RA, and targeting PD-1+NK cells may be a potential therapeutic approach for RA.

Keywords: Rheumatoid arthritis, Programmed cell death-1, Natural killer cell, DAS-28

Analyzing the Role of Inflammation-Related circRNA in the Pathogenesis of MS Using an Animal Model

Leila Mohamed Khosroshahi[1], Farshid Noorbakhsh[1]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Background: Multiple sclerosis is an autoimmune and inflammatory disease of the nervous system. Studies have shown that circular RNAs regulate gene expression in different cells, including leukocytes, in physiological and pathological conditions. In this work, we investigated the expression of inflammation-related circRNAs in inflamed CNS tissue in EAE mice.

Methods: Inflammation-related circRNAs were identified by literature reviews and database searches. Divergent and convergent primers were designed by circPrimer software. EAE was induced in 30 C57/BL6 female mice, and spinal cord tissues were isolated at different time points after disease induction. To perform in vitro stimulation experiments, splenocyte cultures were prepared from 6- to 8-week-old C57BL/6 mice. RT-PCR analyses were used to detect and quantify the expression of circRNAs as well as their corresponding linear forms.

Results: Enhanced expression of inflammation-related genes was detected in EAE lumbar spinal cords at acute and chronic phases of the disease and activated splenocytes. Examining the expression of circRNAs (circ-SNX5, HIVEP1, MALAT1, ARID1A, RasGEF1b, HIPK3, ZC3H4, Samd4, TGFBR1, KCNT2, CDR1, IL15RA) in lumbar cord tissues from EAE mice showed significant alterations at the acute and chronic phases of disease compared with control mice. Sanger sequencing of the PCR product of divergent primers confirmed the presence of a back splice site in circRNAs. The expression levels of circRNAs were positively correlated with the expression of inflammatory cytokines

Conclusion: Our data point to the role of inflammation-related circRNA in autoimmune neuroinflammation. CircRNAs that are associated with EAE progression at different clinical phases might provide potential therapeutic targets for regulating inflammation in the context of autoimmune neuroinflammation.

Keywords: circRNA, Neuroinflammation, Multiple sclerosis, Experimental autoimmune encephalomyelitis, T cell differentiation

Analyzing The Role of Rasgef1b circRNA in Macrophage Activation and Its Expression in EAE Animal Model

Mohammad Reza Zabihi[1], Leila Mohamed Khosroshahi[1], Farshid Noorbakhsh[1]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Background: Multiple sclerosis (MS) is an autoimmune and inflammatory disease of the central nervous system (CNS). Different immune cells, especially T cells and macrophages are involved in MS pathogenesis. Former studies have shown that circular RNAs regulate different cells' gene expression during various situations, such as physiological and pathological conditions. "circRasGEF1B" is a circRNA involved in macrophage activation. The current study investigates the role of this circRNA in the activation and polarization of macrophage and its expression in the CNS in EAE mice.

Methods: C57/BL6 female mice were utilized to induction of EAE model. Spinal cord tissues and the lower lumbar cord were isolated from EAE and control animals at different time points after model induction. Divergent primers were designed for the circular form of circRasGEF1B using circPrimer software. circRNA expression levels were examined by real-time RT-PCR using divergent primers. Bone marrow-derived macrophage (BMDM) cultures were prepared and differentiated into M1 and M2 subtypes. Real-time RT-PCR was performed on the RNA extracted from BMDMs in resting conditions following activation and polarization to M1 and M2 phenotypes. Sanger sequencing was performed for circRNA PCR products to validate back-splicing junction (BSJ) amplification.

Results: Our qPCR results in EAE tissues demonstrate that circRasGEF1b is downregulated significantly in acute and chronic EAE versus normal tissues. However, circRasGEF1b is upregulated significantly in M1 macrophages versus M0 and M2. circRasGEF1b expression negatively correlated with IFNϒ, TNFα, IL1, IL6, and IL12 in EAE tissues. Moreover, the expression of circRasGEF1b in polarized macrophages displayed a positive correlation with TNFα, IL1, IL6, and iNOS. Also, a negative correlation was observed between circRasGEF1b and Mrc.

Conclusion: circRasGEF1b expression is dysregulated in polarized and activated macrophages and in spinal cord tissues the EAE model.

Keywords: Experimental Autoimmune Encephalomyelitis, Macrophage Activation, Circular RNA, Multiple Sclerosis, RasGEF1b

Association of Human Endogenous Retrovirus-W (HERV-W) Copies with Pemphigus Vulgaris Patients

Hanieh semsari[1], Abbas karimi[2], Esmaeil Babaei[1], Mohammadreza Ranjkesh[3], Nafiseh Esmaili[4], François Mallet[5]

1. Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.
2. Department of Molecular Medicine, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.
3. Department of Dermatology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
4. Autoimmune Bullous Diseases Research Center, Tehran University of Medical Sciences, Razi Hospital, Tehran, Iran
5. EA 7426 Pathophysiology of Injury-Induced Immunosuppression, Edouard Herriot Hospital, University of Lyon1-Hospices Civils de Lyon-bioMérieux, 5 Place d'Arsonval, Lyon Cedex 3, Lyon, France

Background: Pemphigus is classified as an autoimmune group that results in blisters and skin lesions caused by IgG antibodies and loss of cell junctions in the epidermis. Sequences of human endogenous retroviruses (HERVs) and their products (RNA, cytosolic DNA, and proteins) can modulate the immune system and contribute to autoimmunity. Previous studies show that the expression of HERV-W env is increased in some autoimmune diseases including MS, and psoriasis, SLE, pemphigus, but to what extent HERV-W env variants may be involved in the pathogenesis of pemphigus is still unclear. The present study aimed to investigate the relative levels of HERV-W env DNA copies in patients and control groups.

Methods: 31 patients and healthy controls who were matched in terms of age and gender participated in this study. Using specific primers and the qPCR method, relative DNA levels of HERV-W env copies in peripheral blood mononuclear cells (PBMCs) of patients and control groups were investigated.

Results: Based on the results of statistical analysis, the relative DNA levels of HERV-W env copies in patients were significantly higher than the control group (1.67±0.86 vs. 1.17±0.75; p=0.02). Also, there was a significant difference between HERV-W env versions of male and female patients (p=0.001). HERV-W env copy number with disease onset (p= 0.19 (p= 0.19)) and serum levels of Dsg-1 (p=0.86) and Dsg-3 (p=0.76) were also analyzed separately. No significant difference was found.

Conclusion: Our findings showed that there is a positive correlation between HERV-W env copies and the pathogenesis of pemphigus. It will be an interesting attempt to evaluate the HERV-W env copies of pemphigus vulgaris patients for diagnosis and response to treatment goals, but the correlation between clinical severity score and HERV copies -W env in PBMCs as a biomarker for PV needs further studies.

Keywords: Human endogenous retroviruses (HERV), Autoimmune Diseases, Pemphigus, Env Gene

Changes in the Expression of Synovialin, miR-125a-5p, and miR-19b-3p in Peripheral Blood of Patients with Rheumatoid Arthritis during Treatment with Conventional DMARDs and Methylprednisolone

Nagin Karamali[1], Seyed Askar Roghani[1], Mehran Pournazari[1], Amirhossein Mardi[3], Amin Kamrani[3], Alireza Rezaiemanesh[1]

1. Kermanshah University of Medical Sciences, Kermanshah, Iran.
3. Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Disease-modifying antirheumatic medications (DMARDs) are immunosuppressive and immunomodulatory pharmaceuticals approved for the management of several connective tissue disorders including rheumatoid arthritis (RA). On the other, SYVN1, an endoplasmic reticulum (ER)-resident E3 ubiquitin ligase, plays a crucial role in the pathophysiology of RA, together with SEL1L. The purpose of this study was to determine the gene expression level of peripheral blood SYVN1 and SEL1L and their predicted upstream ncRNAs after treatment with DMARDs and methylprednisolone.

Methods: 25 newly diagnosed RA patients enrolled to receive methylprednisolone and conventional DMARDs (cDMARDs) for six months. Using qRT-PCR, the expression of the genes SYVN1 and SEL1L in peripheral blood as well as predicted regulatory axis NEAT1, miR-125a-5p, and miR-19b-3p were assessed before and after therapy. Additionally, we analyzed variations between newly diagnosed patients and healthy controls (HCs). Statistical analyses were carried out to ascertain if ncRNAs with SYVN1-SEL1L and the clinical parameters of RA were correlated.

Results: Expression levels of NEAT1 (p=0.0001), miR-19b-3p (p=0.007), miR-125a-5p (p=0.005), and SYVN1 (p=0.036) were all higher in newly diagnosed patients compared to HCs. miR-125a-5p, miR-19b-3p, and SYVN1 were also overexpressed after treatment (p=0.001, p=0.001, and p=0.005, respectively). NEAT1 and SYVN1 exhibited a positive correlation, while miR-125a-5p and anti-cyclic citrullinated peptide had a negative correlation.

Conclusion: Our findings demonstrated that although cDMARDs therapy reduces clinical symptoms of RA, it may have the opposite impact on the gene-related expression of ER stress. Also, the difference in ncRNA expression may serve as significant markers for monitoring disease activity and predicting therapy outcomes in RA patients.

Keywords: Rheumatoid arthritis, ERAD, ncRNA, Therapeutic

Chlamydia Peumonia Infection and Risk of Multiple Sclerosis: A Meta-Analysis

Delaram Arjmandi[1], Ali Rostami[2]

1. Immunoregulation Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
2. Infectious Diseases and Tropical Medicine Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

Background: This meta-analysis study aimed at the assessment of the actual involvement of Chlamydia pneumoniae (Cpn) in multiple sclerosis (MS) developments.

Methods: We undertook a literature search of international scientific databases including PubMed/Medline, Scopus, Embase, and Web of Science as of 10 December 2022. We used a random-effects meta-analysis model (REM) to generate the pooled odds ratio (OR) and 95% confidence intervals (CIs). Heterogeneity was calculated using the I2 statistic. Sensitivity, subgroup, and meta-regression analyses were applied to assess the effects of study characteristics and socio-demographic variables on pooled OR.

Results: We identified 37 studies comprising 51 datasets that satisfied the inclusion criteria. Considering diagnostic methods for Cpn, 26 and 25 datasets used PCR- and serological-based methods, respectively. In PCR-based datasets, REM showed a significant positive association between Cpn infection and the development of MS (OR, 5.29; 95%CI, 3.12–8.97), while a non-significant positive association was achieved in serological-based datasets (OR, 1.34; 95%CI, 0.88 –2.03). In subgroup analyses on PCR-based datasets, results were significant for both CSF (OR, 5.70) and serum (OR, 4.84) samples; both healthy (OR, 16.11) and hospital-based (OR, 2.88) controls; and both moderate (OR, 5.14) and high (OR, 5.48) quality studies. In serological-based datasets, only those that used CSF samples yielded significant results (OR, 3.41).

Conclusions: Our findings verify the significant positive relationship between Cpn infection and MS. We advocate prospective cohort studies with lifelong follow-ups and also experimental studies to better understand the role of Cpn in MS development.

Keywords: Multiple sclerosis, Chlamydia pneumonia, association, meta-analysis

Circulatory Level of Cadmium, Lead, and Iron in Multiple Sclerosis Patients; A Systematic Review and Meta-Analysis

Alireza Kooshki[1], Samaneh Nakhaee[2], Alireza Amirabadi Zadeh[2], Omid Mehrpour[3]

1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran
2. Medical Toxicology and Drug Abuse Research Center (MTDRC), Birjand University of Medical Sciences, Birjand, Iran
3. Data Science Institute, Southern Methodist University, Dallas, Texas, USA

Background: The role of trace elements and heavy metals on human health and risk factors of various diseases has been a subject of discussion for a long time. Multiple sclerosis as a common autoimmune disease has been no exception in this. MS is mostly known as an inflammatory demyelination disease that involves individuals ranging between 20 to 40 years old. In this systematic review and meta-analysis, we investigate the coloration between cadmium (Cd), lead (Pb), and iron (Fe) levels in MS patients compared to healthy controls in various biological samples.

Methods: In this systematic review and meta-analysis, we searched 4 various databases/search engines; Web of Science, PubMed, Scopus, and Google scholar till March 12th, 2022. After data scanning, unrelated data were excluded and related studies were included in our study. The meta-analysis was performed by STATA version 17.0. 35 studies were included in our systematic review.

Results: In our meta-analysis Cd levels in MS patients were significantly higher compared to controls. (Hedges' g: 1.20, 95% CI: 0.13, 2.27, p=0.028) but for lead and iron, no significant difference was observed. (Respectively; Hedges' g: 1.35, 95% CI: -0.52, 3.22, p>0.05 and Hedges' g: -0.55, 95% CI: -1.16, 0.05, p=0.074. subgrouping was also implied by Body fluid type, Assessment Method of the element, and development of countries. In all three elements, developing countries showed a wider confidence interval compared to developed countries. This meta-analysis showed that Cd level in MS patients is relatively higher than controls in biological samples and Iron and Lead do not change significantly in MS patients.

Conclusion: Considering the great incidence of MS and the possible correlation between this MS and toxic metals exposure, we should have more protected encounters with contaminated environments.

Keywords: Multiple Sclerosis, Iron, Cadmium, Lead

Combination of Aptamers with Gold Nanoparticles Ameliorated Psoriatic Lesions in Imiquimod-Induced Psoriasis Mouse Model

Razia Khorrami[1], Saeide Sadat Shobeiri[1], Zahra Emami Koorandeh[1], Mojtaba *Sankian*[1]

1. Mashhad University of Medical Sciences, Mashhad, Iran.

Background: Psoriasis is an inflammatory and chronic skin disease in which inflammatory cytokines stimulate keratinocytes, neutrophils, endothelial cells, fibroblasts, and so on. Recent studies have shown that blocking inflammatory cytokines can be used to treat psoriasis. Our previous experiments in vitro and in vivo showed that anti-IL17A M2 aptamers inhibit the action of IL -17. To increase the molecular size and anti-inflammatory effect of M2 aptamer, we developed a gold nanoparticle linked to an anti-IL17A aptamer for the treatment of psoriasis in an imiquimod (IMQ) mouse model.

Methods: We divided C57BL/6 mice into eight groups. Imiquimod (IMQ) cream and petrolatum (Vas) were applied to the dorsal skin of the mice as IMQ-induced psoriasis and negative control. The control group received dexamethasone (Dexa) as an intraperitoneal injection. 10 minutes before IMQ treatment, the hydrogel containing aptamer M2 (350 pmol), aptamer M2 (44 pmol), aptamer M2 (350 pmol) combined with gold nanoparticles, and aptamer M2 (44 pmol) combined with gold nanoparticles was topically applied to the dorsal skin of the mice. A group that received only gold nanoparticles served as a control group. The psoriatic lesions were evaluated by histology, clinical factors, and Psoriasis Area Severity Index (PASI).

Results: Evaluation of the PASI scores showed that M2 (44 pmol), M2 (350 pmol), Nanogod-M2 (350 pmol), and Nanogod-M2 (44 pmol) had scores of 2.18, 2.4, 0.58, 0.56. In addition, the aptamer M2 (44 pmol) associated with the gold nanoparticles showed a more significant decrease than the aptamer M2 (350 pmol).

Conclusion: According to our results, the combination of aptamer M2 with gold nanoparticles enhanced the anti-inflammatory effect of M2, and improve the IL-17 blocking effect of this aptamer.

Keywords: IL-17, psoriasis, autoimmunity, PASI score, aptamer

Comparison of the PD-1 Expression Level in Peripheral Blood of Multiple Sclerosis and Neuromyelitis Optica Patients

Melika Gorgani[1], MirHadi Jazayeri[1]

1. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Programmed cell death-1 (PD-1) is one of the important co-inhibitory receptors in maintaining tolerance and inhibiting the proliferation and activity of activated immune cells. PD-1: PD-L pathway enhances regulatory T cells proliferation and inhibition of autoreactive T cells and regulates central and peripheral tolerance. This pathway has affected various aspects of the immune system and is of particular importance in a variety of diseases, such as autoimmune diseases. Multiple sclerosis (MS) and Neuromyelitis Optica (NMO) are neurological disorders characterized by inflammation, demyelination by the immune system, and axonal and neuronal damage in the CNS. The clinical manifestations of NMO are very similar to MS and lead to a misdiagnosis. Therefore, the existence of specific diagnostic markers is of particular importance for correct diagnosis.

Methods: Here, we examined the expression of the PD-1 gene in 40 MS patients, 20 NMO patients, and 15 healthy individuals. Thus, after RNA extraction from human blood and cDNA synthesis, gene expression of PD-1 was investigated using quantitative real-time PCR technique.

Results: The results show that the PD-1 mRNA expression in the peripheral blood of the MS group was significantly increased in comparison to that of the NMO group and healthy group ($p=0.0008$, $p= 0.0024$, respectively). However, there was no significant difference in PD-1 mRNA expression between the NMO group and the healthy group.

Conclusion: The similarities in the clinical manifestations between MS and NMO make the correct differentiation between these two diseases challenging. In this study, it was shown that the difference in PD-1 gene expression between these two diseases can be considered a diagnostic biomarker.

Keywords: Multiple sclerosis, Neuromyelitis optica, Misdiagnosis, PD-1, PD-L, T cell, Gene expression

Dysregulated LKB1-AMPK Signaling During Bleomycin-Induced Pulmonary Fibrosis Development

Marjan Taherian[1], Paria Bayati[1], Nazanin Mojtabavi[1]

1. Department of Immunology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Idiopathic pulmonary fibrosis (IPF) is a progressive and devastating lung disease with complex pathogenesis and no effective therapy. Recent experiments have highlighted the significance of the LKB1-AMPK pathway in fibrosis; however, its role in the IPF has not been revealed, nor has the relationship between this pathway and autophagy in pulmonary fibrosis. Therefore, this study aimed to investigate the potential involvement of LKB1-AMPK signaling in bleomycin-induced pulmonary fibrosis.

Methods: Male C57BL/6 mice were intratracheally administrated with Bleomycin (BLM) or phosphate-buffered saline (PBS) and were assessed on day 21 to remove their lungs. The induced lung fibrosis was evaluated by histological examinations as well as hydroxyproline assay. The expression of target genes was investigated by qPCR.

Results: Mice that received BLM showed inflammatory and fibrotic changes in their lungs that resembled IPF. Gene expression analyses revealed a significant up-regulation of TGF-β1, SNAIL1, ZEB1, RPTOR, and Atg13, along with a significant down-regulation of LKB1, AMPK, and ULK1 expression in the BLM group.

Conclusion: Our findings demonstrate dysregulation of LKB1-AMPK signaling during the development of BLM-induced lung fibrosis in mice, which is suggestive of defective autophagy.

Keywords: Idiopathic pulmonary fibrosis (IPF), TGF-β signaling, LKB1, AMPK, RPTOR, Autophagy

Effect of Spirulina on Type 1 Diabetes

Nasrin Omidi[1], Nakisa Sohrabi Haghdoost[2], Parya Mirhosseini[3], Amir Esmail Razmjou[4]

1. Department of Biology, Unit Miyaneh Islamic Azad University, Miyaneh, Iran
2. Department of Pathobiology, School of Veterinary Medicine, Science, and Research Branch, Islamic Azad University, Tehran, Iran
3. Department of Veterinary Clinical Sciences, Tabriz University Campus, University of Tabriz, Tabriz, Iran
4. Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran

Background: Type 1 diabetes is an autoimmune disease that occurs as a result of the destruction of insulin-producing beta cells in the pancreas. It has been suggested that spirulina, blue-green algae with immunomodulatory properties, may have a beneficial effect on the immune system and could potentially be used as a complementary therapy for individuals with autoimmune diabetes.

Methods: To examine the potential effects of spirulina on autoimmune diabetes, a randomized, double-blind, placebo-controlled study was conducted. Fifty individuals with newly diagnosed type 1 diabetes were randomly assigned to receive either spirulina supplementation or a placebo for 12 weeks. Fasting glucose levels, hemoglobin A1C (HbA1c) levels, and autoantibodies against pancreatic islet cells were measured at baseline and the end of the study.

Results: At the end of the 12-week intervention, there were no significant differences in fasting glucose levels or HbA1c levels between the spirulina and placebo groups. However, there was a significant decrease in the levels of islet cell autoantibodies in the spirulina group compared to the placebo group ($p<0.05$).

Conclusion: In this study, spirulina supplementation did not have a significant effect on glucose control in individuals with autoimmune diabetes. However, the observed decrease in autoantibody levels suggests that spirulina may have a potential role in the prevention or treatment of autoimmune diabetes. Further studies are needed to confirm these findings and to elucidate the mechanisms underlying the observed effects of spirulina on the immune system.

Keywords: Spirulina, Type 1 diabetes

Efficacy of DMARDs and Methylprednisolone Treatment on The Gene Expression Levels of HSPA5, MMD, And Non-Coding RNAs MALAT1, H19, miR-199a-5p, And Mir-1-3p, In Patients with Rheumatoid Arthritis

Zahra Mahmoudi[1], Negin Karamali[1], Seyed Askar Roghani[2], Shirin Assar[3], Mehran Pournazari[3], Parviz Soufivand[3], Farhad Salari[1], Alireza Rezaiemanesh[1]

1. Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
3. Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease with chronic inflammation characterized by joint damage and even extra-articular involvement. In this study, the gene expression levels of MALAT1, H19 and their possible downstream microRNAs, miR-199a-5p, miR-1-3p, and the predicted targets of these miRNAs, HSPA5 and MMD, were examined.

Methods: Twenty-five newly diagnosed RA patients and 25 healthy individuals were included. Patients were treated with conventional disease-modifying antirheumatic drugs (DMARDs) and Methylprednisolone (mPRED) for six months. Blood samples were obtained from healthy controls and patients (before and after treatment). After RNA extraction, the RT-qPCR technique was used to evaluate the expression level of the studied genes.

Results: Data showed that the expression level of MALAT1, H19, miR-199a-5p, and miR-1-3p was significantly higher in the newly diagnosed patients with RA than in the healthy subjects, but the increase in the expression level of HSPA5 and MMD genes in the new cases was not significant compared to the healthy controls. After treatment, except for the expression level of lncRNAs, the expression level of miRNAs, HSPA5, and MMD significantly increased. Based on ROC curve analysis of MALAT1, H19, miR-199a-5p, and miR-1-3p have a high ability to identify patients from healthy individuals (AUC = 0.986, AUC = 0.995, AUC = 0.855, AUC = 0.675, respectively).

Conclusion: MALAT1 and H19 can be considered potential biomarkers for the discrimination between RA patients and controls. DMARDs plus mPRED therapy do not have a desirable effect on reducing inflammatory responses and ER stress.

Keywords: Rheumatoid arthritis; DMARDs; ncRNAs; MMD; HSPA5

Evaluation of Inflammatory Genes mRNA Expression Level in a Mouse Model of Psoriasis Treated with Dexamethasone and Anti-Interleukin-17 Aptamer

Zahra Emami Koorandeh[1], Saeide Sadat Shobeir[1], Razia Khorrami[1], Mojtaba Sankian[1]

1. Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Psoriasis is an inflammatory skin disease with clinical manifestations such as redness, scaling, and thickening of the skin mediated by the immune system. Studies have shown that psoriasis is associated with increased expression of inflammatory genes. In this study, we will evaluate the mRNA expression level of Kynureninase (Kynu), Cathelicidin, β-defensin2, and Proenkephalin (Penk) to find a suitable marker for the treatment responsiveness of the psoriasis mouse model.

Methods: The C57BL/6 mice were divided into four groups. Vaseline and imiquimod (IMQ) cream were applied to the dorsal skin of the mice as negative control and IMQ-induced psoriasis (3 groups), respectively. Two types of treatment, dexamethasone, and anti-IL17 aptamers-containing hydrogel were administered 10 minutes before IMQ treatment. The psoriatic lesions were evaluated by clinical symptoms, Psoriasis Area Severity Index (PASI) score, and histology. In addition, mRNA expression levels of Kynu, Cathelicidin, β-defensin2, and Penk were assessed with SYBR green real-time PCR.

Results: The IMQ-treated area of non-treated mice showed 5.01, 3.02, 3.43, and 2.42-fold changes in mRNA expression levels of Kynu, β-Defensin2, Cathelicidin, and Penk genes, respectively, compared to the Vaseline group and treated mice.

Conclusion: The changes in mRNA expression levels of Kynu and Cathelicidin can be considered appropriate criteria for treatment response in an IMQ-induced psoriasis mouse model.

Keywords: IL-17, psoriasis, autoimmunity, PASI score, aptamer, Kynu, Cathelicidin, β-defensin2, Penk

Evaluation of Oligoclonal Bands and Neuromyelitis Optica by Exploiting Isoelectric Focusing and Immunofluorescence Studies for Accurate Diagnosis in Multiple Sclerosis Patients in Tehran

Mahsa Tahsili[1]

1. PNU of Taft, Iran

Background: Multiple Sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system that causes various clinical symptoms, which may overlap the symptoms of other disorders; such as Neuromyelitis Optica (NMO). The proper differential diagnosis is necessary for accurate treatment. The presence of oligoclonal bands (OCB) in the cerebrospinal fluid (CSF), which are not present in the serum, is the main laboratory assay in the differential diagnosis of MS from NMO. On the other hand, the presence of serum autoantibodies against Aqa4 protein in NMO is another laboratory characteristic, not present in MS.

Methods: The present study reports, for the first time, the relationship between OCB and NMO analysis, carried out by Isoelectric focusing (IEF) and immunofluorescence (IF), respectively, in MS patients. In this study, 20 MS patients and 20 healthy individuals were selected according to the diagnostic criteria in Tehran, Iran. In addition, the participants were gender- and age-matched.

Results: The results demonstrate a significant difference between the case group and the control, regarding the presence of OCB in CSF, while the results of the NMO test did not show a statistically significant difference between the case group, and positive and negative controls. Also, our study results showed that 75% of the MS diagnosis for both tests were negative.

Conclusion: Overall, by consideration of the results can be concluded that in addition to the clinical examinations and MRI, laboratory test for differential diagnosis seems necessary. However, more research is needed on the specificity and sensitivity of different laboratory methods.

Keywords: Multiple sclerosis, Oligoclonal bands, Cerebrospinal fluid, Immunofluorescence, Isoelectric focusing

Evaluation of the Different Approaches in the Treatment of EAE Animal Model: Caspr- Conjugated and Exosome-Labeled Gold Nanoparticles

Shirin Sadat Taghizadeh[1], Morteza Motallebnezhad[2], Mir Hadi Jazayeri[2]

1. Faculty of Medical Sciences, Islamic Azad University, Shahrood Branch
2. Department of Immunology, School of Medicine, Iran University of Medical Sciences

Background: Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system which is increasing worldwide. Although immunosuppressive agents are used for the treatment of MS disease, nevertheless the lack of a non-toxic and efficient therapeutic method is perceptible. Hence, this study aims to evaluate the effect of Contactin-associated protein (Caspr) antibody-, polyethylene glycol (PEG) - and exosome combined gold nanoparticles (GNPs) in comparison to Glatiramer acetate as a selective treatment of MS disease in the experimental autoimmune encephalomyelitis (EAE) mouse model.

Methods: EAE was induced in female C57BL/6 mice and 25-day treatment with anti-Caspr-, PEG-, and exosome combined GNPs was evaluated. Histopathological examination of the spinal cord, regulatory T cells as well as inflammatory pathways including IFN-ɣ and IL-17, and mir-326 were investigated.

Results: The results showed the severity of MS symptoms was significantly decreased in all treated groups. Histological examination of the spinal cord indicated reduced demyelination and immune cell infiltration. Besides, regulatory T cells were significantly increased following all treatments. Remarkably, the cytokine levels of IFN-ɣ and IL-17 as well as mir-326 altered in treated groups.

Conclusion: Taken together, the obtained findings demonstrate that the administration of anti-Caspr-, PEG- and exosome combined GNPs can be considered a potential treatment for MS disease.

Keywords: Multiple sclerosis, Experimental autoimmune encephalomyelitis, Gold nanoparticle, Contactin-associated protein, Polyethylene glycol, Exosome

Evaluation of Tumor Necrosis Factor-A and Nuclear Factor-Kb in Patients with Newly Diagnosed Type 2 Diabetes Mellitus

Zahra Sardarmelli[1], Vida Sheikh[2], Ghasem Solgi[1], Mahdi Behzad[1]

1. Department of Immunology, Hamadan University of Medical Sciences, Hamadan, Iran
2. Department of Internal Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Background: Type 2 diabetes mellitus (T2DM) is linked to chronic inflammation. Tumor necrosis factor-α (TNF-α) and nuclear factor-κB (NF-κB) are commonly expressed by immune cells. These factors are associated with inflammation and an alteration of them has been involved in a variety of inflammatory disorders. In this study, the serum levels of TNF-α and gene expression of NF-κB were investigated in T2DM patients compared to healthy controls.

Methods: Blood samples from 35 newly diagnosed patients with T2DM and 35 healthy controls were collected. The serum concentration of TNF-α was determined by enzyme-linked immunosorbent assay (ELISA). Peripheral blood mononuclear cells (PBMCs) were purified and cDNA was generated from each donor. The expression of NF-κB and GAPDH reference gene was determined with SYBR green real-time PCR method. mRNA levels were calculated by the 2-ΔCq formula. Analyses were done by independent-sample t-test.

Results: Data showed that the serum levels of TNF-α in diabetes patients were significantly higher than in healthy controls ($p<0.001$). Similarly, enhanced gene expression levels of NF-κB were observed in T2DM patients ($p<0.001$).

Conclusion: Abnormal expression of TNF-α and NF-κB may contribute to diabetes pathogenesis and these factors could be suggested for a therapeutic target in T2DM.

Keywords: Gene, Cytokine, Type 2 diabetes mellitus

Flow Cytometric Analysis of Follicular T Helper Lymphocytes and Monocytes in Multiple Sclerosis Patients Being Treated with Rituximab in Comparison to Newly Detected Subjects and Healthy Controls

Sheida Yahyazadeh[1], Nafiseh Esmaeil[2], Vahid Shaygan-nejad[3], Omid Mirmosayyeb[4]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Neurology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Neurology, Jacobs School of Medicine and Biomedical Sciences, University of Buffalo, Buffalo, New York, United States

Background: Multiple sclerosis (MS) is a debilitating neurodegenerative disease that mostly impacts young individuals. Historically, it has been categorized as a demyelinating T cell-associated autoimmune disorder. Rituximab (RTX), a B cell-depleting monoclonal antibody, has been reported to be therapeutically effective in MS management. Moreover, it is proposed to have decreasing effects on the T cell population, as well. Herein, we evaluated the effects of RTX treatment on follicular T helper cells (Tfh) and monocytes in MS patients versus newly-detected subjects and healthy controls.

Methods: MS patients from the MS clinic of Kashani Hospital, Isfahan, Iran, were included in this investigation. 30 subjects were classified as the RTX-treated group and the other group (n=30) was composed of newly diagnosed patients, who did not receive any immunosuppressive drug. 30 age-and-sex-matched healthy individuals were also enrolled as control subjects. Blood specimens were collected from all participants. Peripheral blood mononuclear cells (PBMCs) were then isolated from whole blood and incubated with FITC-anti-CD4, PE-anti-CD45RA, PerCP-anti-CXCR5, FITC/PE-anti-CD4/8, FITC/PE-anti-CD14/45, FITC/PE-anti-CD3/HLA-DR, FITC/PE/PerCP-anti-CD4/25/3, FITC-anti-CD19 antibodies for 20 min. at 4 °C in the dark. Whole blood was also lysed for flow cytometric analysis of CD45+CD14+ monocytes. A BD FACSC Calibur cytometer was used for flow cytometry, and data were analyzed using Flow jo software 10.

Results: RTX therapy caused a decline in Tfh cells. Furthermore, a statistically significant decrease was detected in CD3+HLA-DR+ and CD3+CD4+CD25+ T cells in RTX-treated subjects in comparison to newly diagnosed cases and healthy controls. We also understood that CD45+CD14+ monocytes were decreased in those who received RTX compared to healthy controls.

Conclusion: According to our findings, RTX therapy has the potential of reducing Tfh cells, monocytes, and T cells priming, and therefore fewer T cells would be activated post-treatment. Hence, regarding the B cells-Tfh cells network, targeting Tfh cells could be considered a potential approach in MS therapy.

Keywords: Multiple sclerosis, Rituximab, T lymphocytes, Monocytes

Following the Children and Adolescents with Systemic Lupus Erythematosus during the COVID-19 Outbreak

Zahra Kanannejad[1], Soheila Alyasin[1], Maryam Babaei[1]

1. Allergy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: This study investigated the effect of the COVID-19 pandemic on pediatric patients with systemic lupus erythematosus (SLE).

Methods: The telephone survey was administered by conducting interviews with the parents of 71 patients.

Results: Regarding the general population followed in our study, only 1.4 % (1/71) tested positive for SARS-CoV-2.

Conclusion: Our data, although still limited in number, did not result in a high incidence of COVID-19 and a worse outcome of infection for pediatric and adolescent patients with systemic lupus erythematosus.

Keywords: COVID-19, Pandemics, Lupus Erythematosus Systemic

Helicobacter Pylori Infection and Multiple Sclerosis: An Updated Meta-Analysis

Delaram Arjmandi[1,2], Ali Rostami[1,2]

1. Infectious Diseases and Tropical Medicine Research Center, Health Research
2. Institute, Babol University of Medical Sciences, Babol, Iran

Background: There is considerable controversy around the question of whether Helicobacter pylori (H. pylori) infection has a protective or causative role in the development of multiple sclerosis (MS). This study evaluated published information to assess the association between H. pylori infection and MS.

Methods: We conducted a comprehensive systematic review of relevant observational studies in international databases. A random-effects model was used to calculate pooled odds ratio (OR) and 95% confidence interval (CI). I2 statistic was used to assess the between-study heterogeneity. Subgroup and meta-regression analyses were applied to identify the source of heterogeneity.

Results: In total, 22 studies (25 datasets) were eligible for the meta-analysis; 17 datasets had prevalence data and eight datasets had data on the mean titer of anti- H. pylori IgG. The pooled prevalence of H. pylori was 44.1% (908/2606) in the MS patients and 46.1% (1016/2200) in the controls, indicating a non-significant protective effect of H. pylori on MS (OR, 0.82; 95%CI, 0.58–1.17). In the subgroup analysis, studies that used ELISA yielded a significant protective association (OR, 0.59; 95%CI, 0.46–0.77), while a significant positive association (OR, 5.75; 95%CI, 2.40–13.76) was found in studies that used histological methods.

Conclusions: Our findings do not support the hypothesis that H. pylori infection represents a protective factor against the development of MS; however, the results varied depending on the diagnostic method(s). Further studies are needed utilizing accurate diagnostic methods to elucidate the association between active H. pylori infection and MS.

Keywords: Multiple sclerosis, Helicobacter pylori, association, meta-analysis

IFN-Γ Functions as a Double-Edged Sword on the Inflammation and Fibrosis in the Pathogenesis of Systemic Sclerosis

Mohsen Rokni[1], Elham Farhadi[2], Hoda Kavosi[2], Maryam Akhtari[2], Samaneh Enayati[2], Mina Sadeghi Shaker[1], Mahdi Mahmoudi[2], Mohammad Vodjgani[1]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Systemic sclerosis (SSc) is an autoimmune systemic disease that is characterized by immune dysregulation, inflammation, vasculopathy, and finally, fibrosis. Tissue fibrosis plays an important role that can affect several organs, such as the dermis, joints, gastrointestinal tract, lungs, and heart. Dysregulation of type I interferon (IFN I) involves in the SSc pathogenesis, and IRF-1 has been indicated as the main regulator of type I IFN. This study aimed to clarify the role of interferon-gamma (IFNγ) and dexamethasone (DEX) on the IRF-1gene expression and genes involved in the inflammation and fibrosis process in SSc pathogenesis.

Methods: A total of 10 SSc patients (Diffuse form) and 10 unaffected control dermis biopsies were obtained to determine IRF-1 effects on inflammation and fibrosis. Fibroblasts were treated with IFNγ, which acts like an inducer, and DEX as an inhibitor of IRF-1 expression. The expression level of genes involved in the fibrosis and inflammation process (Such as IRF1, IL6, COL1A1, COL1A2, and FN1) was evaluated by RT-qPCR.

Results: FN1 mRNA level was over-expressed in explanted SSc dermal fibroblasts compared with unaffected, matched controls. No statistically significant difference was noticed between the two groups concerning IRF1. Moreover, IRF1 expression was stimulated by IFNγ in dermal fibroblasts (p=0.0004). Specifically, Col1a1, Col1a2, and FN1 mRNA levels were significantly attenuated, and IL6 mRNA levels were significantly increased by IFNγ in SSc dermal fibroblasts. However, FN1 and IL6 mRNA levels were attenuated by DEX in SSc dermal fibroblasts. Our results indicated that combined treatment of IFNγ and DEX mitigates the expression of Col1a1, Col1a2, and FN1 mRNA levels more than alone in SSc dermal fibroblasts.

Conclusion: The dysregulation of IRF-1 in the SSc dermis can play the main role in inflammation, and this evidence indicates IFNγ as a double-edged sword that affects both inflammation (Up-regulated IRF-1 and IL-6) and fibrosis (Down-regulated Col1a1 and FN-1) under different conditions. Therefore, our results suggested that IFNγ in combination with DEX may be a promising new therapeutic strategy for anti-inflammatory and anti-fibrotic properties in SSc patients.

Keywords: Fibrosis, IRF-1, Inflammation, Interferon-gamma, Systemic sclerosis

Immunotherapy Approaches for Neuroimmunological Disorders

Sajad shariat[1], fatemeh jalali[2], Ali Jebali[1]

1. Department of Medical Nanotechnology, Faculty of Advanced Sciences and Technology, Tehran Medical Science, Islamic Azad University, Tehran, Iran
2. Department of Medicine, School of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran

Background: Neuroimmunological diseases and their treatment compromise the immune system, which can increase the risk of infections. Consequently, vaccination to protect against infections is an important part of the clinical management of these diseases. However, a wide range of immunotherapies currently used to treat neurological diseases, particularly multiple sclerosis, can interfere with immune responses to vaccination.

Method: The keywords of Neuroimmunological and immunotherapies have been searched in PubMed and Google Scholar databases between 2020 and 2023.

Results: Immunotherapies used for neuro-immunological diseases affect the immune system in different ways. Their effect on individual risk of infection varies and interacts with vaccine immunology. Both humoral and cellular immune responses to vaccination can be impaired by some immunotherapies, although protection against infection can be achieved in many cases by vaccination. Nevertheless, to improve the care of patients with neuroimmunological diseases, improved knowledge about the interplay between autoimmune disorders, immunotherapies, and immunization with conventional and novel vaccines is needed.

Conclusion: The immune response to vaccination is not yet fully understood, but knowledge is accumulating rapidly.

Keywords: Neuroimmunological disorder, Immunotherapies, Immune system, Vaccine

Increased Expression of Microglia Related Genes, A1AR, CX3CR1, SIRP-A in Brain of IFN-B Treated Experimental Autoimmune Encephalomyelitis (EAE) Induced C57BL/6 Mice

Nadia Sharafkandi[1], Mobin Mohammadi[2], Dariush Haghmorad[3], Ladan bajelan[2], Mohammad Reza Rahmani[2], Abbas Ali Amini[4]

1. Cellular and Molecular the Research Center, Research Institute for Health Development, Kurdistan University of medical sciences, Sanandaj, Iran
2. Department of Immunology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
3. Department of Pathology and Laboratory Medicine, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
4. Cancer and Immunology Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran

Background: Multiple sclerosis (MS) is a complex autoimmune disease of CNS in which the neuronal connection is disrupted. IFN-β is one of the therapeutic immunomodulatory agents widely used in treating MS. Considering the importance of microglial cells in the immunopathogenesis of MS, we studied the effects of IFN-β on microglial-related genes in the brain of EAE-induced mice.

Methods: C57BL/6 mice in the age range of 6-8 weeks were divided into three groups: EAE induced group which was treated with 10000 IU IFN-β s.c every other day; the patient group which EAE induced but received PBS (phosphate buffered saline); and the healthy control group. Twenty days after treatment, the mice were sacrificed and their whole brains were isolated. Brains tissues were used to extract mRNA. After cDNA synthesis, the gene expression of A1AR, CX3CR1, and SIRP-α was evaluated. Finally, a $p< 0.05$ was considered statistically significant.

Results: In patient group, the expression of A1AR (0.0054 ± 0.00213), CX3CR1 (0.0006 ± 0.00018) and SIRP-α (0.04252 ± 0.02506) were in a low level compared to the healthy control (the genes expression in control group were 0.05485 ± 0.02807, 0.32970 ±0.19125, and 0.07965 ± 0.03215 respectively). IFN-β treatment increased the expression of all these three genes compared to the related patient groups (p for A1AR and CX3CR1expression was 0.017 and 0.004 respectively), despite it wasn't significant for SIRP-α (p=0.433). In addition, there was a significant positive correlation between the expression of CX3CR1 and A1AR in all groups (p=0.001).

Conclusion: IFN-β was able to modulate the inflammatory microenvironment of the brain by inducing the microglia anti-inflammatory genes and shifting them to M2 phenotype.

Keywords: IFN-β, EAE, microglia, CX3CR1, A1AR, SIRP-α

Increased Expression of PD-1 on Circulating CD8+ CD3+ T Cells Correlates with EBV Viral Load in MS Patients

Atefeh Najmadini[1], Mohammad Mehdi Mohammadi[1], Ladan Langroudi[1], Hosseinali Ebrahimi Meimand[2], Merat Mahmoodi[1], Moghadameh Mirzaei[3]

1. Department of Medical Immunology, Kerman University of medical sciences, Kerman, Iran
2. Neurology Research Center, Department of Neurology, Kerman University of Medical Sciences, Kerman, Iran
3. Modeling in Health Research Center, Kerman University of Medical Sciences, Kerman, Iran

Background: Multiple sclerosis (MS) is one of the common autoimmune diseases. The exact etiology of MS is still unclear but recent studies have shown the possibility of infectious agent involvement such as Ebstein Bar virus (EBV) in MS pathophysiology.

Methods: In this study, the expression of exhaustion marker, programmed cell death1 (PD-1) CD3+ CD8+ T cells of 25 new case MS patients were compared with healthy donors using flow cytometry. Also, the expression of the EBV gene, BRCF-1, in PBMCs was analyzed using Real-Time PCR.

Results: Results revealed a lower frequency of CD3+ CD8+ T cells in MS patients. Also, increased expression of PD-1 was observed on CTLs which correlated with higher viral loads. Therefore, a lower frequency of CD8+ T cells but a higher exhaustion marker in MS patients reveals a new mechanism of EBV pathogenesis in MS development.

Conclusion: According to the results, exhausted CTLs in patients suffering from MS are increased and thus not able to eradicate virus-infected B cells. Therefore, inefficient immune control of EBV in patients with MS may cause exacerbation of the disease. Future studies on the mechanism of T-cell exhaustion may aid in a better understanding of the disease and the design of effective therapies.

Keywords: multiple sclerosis, Ebstein Bar virus, T cell exhaustion, Programmed cell death 1

Inhibitory Effects of Cold Atmospheric Plasma on Inflammation and Tumor-Like Feature of Fibroblast-Like Synoviocytes from Patients with Rheumatoid Arthritis

Fatemeh Faramarzi[1], Alireza Rafiei[1]

1. Department of Immunology, Faculty of Medicine, Mazandaran University of Medical Science, Sari, Iran

Background: Rheumatoid arthritis (RA) is a chronic, debilitating systemic disease characterized by chronic inflammation and progressive joint destruction. Fibroblast-like synoviocytes (FLSs) are one of the most important players in the pathophysiology of RA, acting like tumor cells and secreting inflammatory cytokines. Previous research has shown that cold atmospheric plasma (CAP) inhibits cancer cells and may have anti-inflammatory properties.

Methods: This study examined the effects of argon plasma jet-produced CAP on the suppression of invasion and inflammation caused by cultured RA-FLS. The findings revealed that CAP reduced cell viability and elevated the percentage of apoptotic RA-FLS by producing reactive oxygen species.

Results: Carboxyfluorescein diacetate succinimidyl ester (CFSE) staining confirmed that CAP could decrease the proliferation of RA-FLS. Furthermore, CAP effectively reduced the production of inflammatory factors (e.g., NF-κB and IL-6) as well as destructive factors like receptor activator of nuclear factor kappa-B ligand (RANKL) and matrix metalloproteinases-3 (MMP-3).

Conclusion: These data suggest that CAP could be a promising treatment for slowing the progression of RA by reducing tumor-like features and inflammation in RA-FLS.

Keywords: Cold atmospheric plasma, Fibroblast-like synoviocytes, Apoptosis, Inflammation; MMP-3, RANKL

Insights into Overlapping of Fibrosis and Cancer: Exploring the Tumor-Related Cardinal Genes in Idiopathic Pulmonary Fibrosis

Marjan Taherian[1], Paria Bayati[1], Mohammad-Ali Assarehzadegan[1], Mansoureh Soleimani[1], Nazanin Mojtabavi[1]

1. Department of Immunology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Emerging evidence suggests that the pathogenesis of idiopathic pulmonary fibrosis (IPF) is quite similar to that of cancer pathogenesis, and several pathways appear to be involved in both disorders. The mammalian target of the rapamycin (mTOR) pathway harbors several established oncogenes and tumor suppressors. The same signaling molecules and growth factors, such as Vascular endothelial growth factor (VEGF), contributing to cancer development and progression play a part in fibroblast proliferation, myofibroblast differentiation, and the production of extracellular matrix in IPF development as well.

Methods: The expression of candidate genes acting upstream and downstream of mTORC1, as well as Vegf and Lrp1 (low-density lipoprotein receptor-related protein 1), was assessed using specific primers and qPCR within the lung tissues of bleomycin-induced IPF mouse models. Lung fibrosis was evaluated by histological examinations and hydroxyproline colorimetric assay.

Results: BLM-exposed mice developed lung injuries characterized by inflammatory manifestations and fibrotic features, along with higher levels of collagen and hydroxyproline. Gene expression analyses indicated a significant elevation of Raptor, Rheb, S6k1, and 4Ebp1, as well as a significant reduction of Vegfa, Tsc2, and Lrp1; no changes were observed in the Tsc1 mRNA level.

Conclusion: Our findings support the elevation of S6K1 and 4EBP1 in response to the TSC/RHEB/TORC1 axis, which profoundly encourages the development and establishment of IPF and cancer. In addition, this study suggests a possible preventive role for VEGF-A and LRP1 in the development of IPF.

Keywords: Cancer; Idiopathic pulmonary fibrosis, mTOR signaling, RAPTOR, S6K1, RHEB, TSC1/2, 4EBP1

Investigating the Possible Association between Toll-Like Receptor 9 (TLR9) Genetic Variants Rs187084 and Rs352140 and Susceptibility to Behcet's Disease in an Iranian Population

Zahra Tadayon[1]

1. Royan Institute, Tehran, Iran.

Background: Behcet's disease (BD) is a multisystem autoimmune disease with unknown etiology which is characterized by relapsing episodes of oral aphthae, genital ulcers, and ocular and skin lesions. Toll-like receptor 9 (TLR9) due to its pro-inflammatory role is of special importance. This study aimed to clarify the possible associations between single nucleotide polymorphisms (SNPs) in the TLR9 gene, including rs352140 C>T and rs187084 A>G, and susceptibility to BD in an Iranian population.

Methods: SNPs were assessed by polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) in 207 patients with BD and 207 age and sex-matched controls.

Results: A significantly higher frequency of rs352140 CT and TT+CT genotypes was observed in the healthy group compared to the patients (p=0.009 and p=0.028; respectively). Further analysis revealed such results in healthy females compared with the female patients (p=0.003 and p=0.009; respectively). In the case of rs187084, significantly higher frequencies of AG and AG+GG genotypes were indicated in healthy individuals compared with the patients (p=0.02 and p=0.018; respectively). rs187084 GG+AG genotype and G allele frequencies were also significantly higher among healthy males compared with the patients (p=0.035 and p=0.045; respectively). Additional analysis proved no significant association between TLR9 SNPs genotypes and disease manifestations among patients.

Conclusion: In conclusion, our results demonstrated that there might be a link between TLR9 gene polymorphisms and resistance to BD among Iranians, however, further analysis using larger sample sizes is required to support our findings.

Keywords: Autoimmunity, Behçet's disease, TLR9, Single nucleotide polymorphisms

Investigation of the Effect of Gliadin Peptides on THP-1 Derived Macrophages Phenotype

Sara Molaaghaee-Rouzbahani[1], Davar Amani[1,2], Mohammad Rostami-Nejad[2]

1. Department of Immunology, School of Medical Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Celiac disease is an immune-mediated disease triggered by the ingestion of gluten. Pathogenesis of celiac disease has been studied and the contribution of each innate and adaptive immune response has been reported, but little is still known about the contribution of macrophages to the onset or maintenance of the disease. Of note, gliadin, the most prominent causative agent of the disease, has been studied to trigger the production of pro-inflammatory cytokines in this disease. However, studies that have investigated the effect of gliadin on macrophages are scarce. Aim: In the present study, we evaluated the response of THP-1-derived macrophage cells to gliadin.

Methods: THP-1 cell line monocytes were used and differentiated into macrophages by phorbol 12-myrisacetat-acetate (PMA). To investigate gliadin peptides' effect on macrophages, in one group, macrophages were treated with 200µg/ml gliadin, in another group macrophages were treated with 50 ng/ml LPS, and in the other group, macrophages were incubated in a culture medium. The expression of TGF-β, IL-6, and IL-10 cytokines at the level of RNA and IL-10 and TNF-α cytokines at the level of proteins were measured by RT-PCR and ELISA techniques, respectively. Moreover, we used CD206 and CD80 markers for evaluating the M1 and M2 phenotypes of macrophages by flow cytometry technique.

Results: This study showed that the incubation of macrophages with gliadin, induced increased pro-inflammatory cytokines expression such as IL-6 and TNF-α production compared to cells that were incubated in a culture medium. Moreover, anti-inflammatory cytokines such as TGF-β and IL-10 were reduced after exposure to gliadin relative to cells incubated in a culture medium. Flow cytometry data showed that treatment of macrophages with gliadin also increased the CD80 marker compared to CD206 relative to the untreated group.

Conclusion: In this study, we provide more evidence that gliadin can induce the production of pro-inflammatory cytokines in macrophages. In addition, this study highlights the importance of the role of the innate immune system in celiac disease.

Keywords: gliadin, Celiac disease, macrophage, innate immune system

Investigation of the Impact of Mesenchymal Stromal Cells Injection in Renal Function of Healthy and Lupus Balb/C Mice Models

Sara Hosseini[1], Seyed-Alireza Esmaeili[1], Mahmoud Mahmoudi[1], Maryam Rastin[2], Abbas Shapouri Moghaddam[3], Akram Hosseinzadeh[1], Nafiseh Tabasi[2], Mahdieh Khazaee[2]

1. Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Immunology Research Center, Bu-Ali Research Institute, Medical School, Mashhad University of Medical Sciences, Mashhad, Iran
2. Immunology Research Center, Bu-Ali Research Institute, Medical School, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Immunology, BuAli Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Systemic Lupus Erythematosus is an autoimmune disease with no definite cure. Mesenchymal stem cell transplantation has emerged as a potential therapy for SLE. Although a majority of SLE patients achieved clinical remission after allogeneic MSC transplantation, autologous MSC transplantation is controversial. In this study, we aim to investigate the potential mechanisms of lupus-BM-MSCs on the sero markers of anti-dsDNA, creatinine, and BUN as well as proteinuria in order to assess renal function during SLE disease in mice.

Methods: 10 female Balb/c mice between 3-5 weeks old were purchased. Lupus disease was induced by intraperitoneal injection of pristane. The body weight, anti-dsDNA, serum creatinine, and BUN levels were measured in two-month intervals. After 6 months, the lupus mice were sacrificed, and lupus MSCs were cultured. In the next phase of the study, 30 mice were divided into five groups, and lupus disease was again induced in the lupus groups. Then, MSCs were intravenously injected into experimental groups. The mice were euthanized and the kidneys of each group were examined histologically by H&E staining and deposition of IgG and C3 by immuno-fluorescent method.

Results: Our results showed that the injection of lupus MSCs into healthy and lupus mice led to a further rise in anti-dsDNA, serum creatinine, BUN and proteinuria levels, IgG and C3 deposition, and further dysfunction of renal tissue in comparison with control groups.

Conclusion: In a nutshell, our results suggest that autologous MSCs may not be eligible for ameliorating renal function of lupus patients due to their immune-regulatory defects in addition to their capability in promoting inflammation which would worsen the disease status.

Keywords: Systemic lupus erythematosus, Mesenchymal stromal cells, Renal function, Balb/c mice

Male Microchimerism in Peripheral Blood from Women with Multiple Sclerosis

Morteza Jafarinia[1], Mina Amoon[2], Ameneh Javid[2], Sina Vakili[3], Erfan Sadeghi[4], Davood Azadi[5], Fereshteh Alsahebfosoul[6]

1. Shiraz Neuroscience Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Biology, Faculty of Science and Engineering, Science and Arts University, Yazd, Iran
3. Shiraz University of Medical Sciences, Shiraz, Iran
4. Fasa University of Medical Sciences, Fasa, Iran
5 Khomein University of Medical Sciences, Khomein, Iran
6. Isfahan University of Medical Sciences, Isfahan, Iran

Background: Multiple sclerosis (MS) is known as an organ-specific T-cell-mediated autoimmune disease of the central nervous system (CNS). Various environmental and genetic variables raise the risk of acquiring MS. Although the precise function of this phenomenon in human health is not completely understood, microchimerism (Mc) has been the subject of extensive research in autoimmune diseases in recent years. The sporadic presence of DNA or cells from one person in the tissue or circulation of another is known as microchimerism. In the present study, we assessed the relationship between MS and fetal microchimerism (FMc) in the province of Isfahan.

Methods: We enrolled 68 women in four groups for this investigation. Two of the groups were MS sufferers with or without a son-bearing pregnancy, while the other two were MS-negative individuals with or without a son-bearing pregnancy. In these groups, the presence of the male genome was evaluated and contrasted. Four milliliters of peripheral blood were collected from all subjects in the tube containing EDTA and DNA was extracted. All participants had a real-time PCR analysis for the DAZ (deleted in azoospermia) region Yq 11.23, a marker for male microchimerism.

Results: According to our findings, compared to the other three groups, MS-positive women who gave birth to sons had a considerably greater percentage of the DAZ (male) genome. Additionally, our findings showed no significant correlation between the percentage of DAZ-positive women and age of onset and Expanded Disability Status Scale (EDSS) score in the patients' group.

Conclusion: For future studies, we suggest enrolling subjects whose MS diagnosis occurred before and after pregnancy with a son. Comparing FMc in these two populations may help us better understand FMc's potential contribution to the later onset of MS.

Keywords: multiple sclerosis, microchimerism, real-time PCR, fetal microchimerism

MiR-155 Targeting T-Cell Immunoglobulin and Mucin Domain 3 (TIM-3) Promote Natural Killer Cell Exhaustion in Ankylosing Spondylitis

Hamed Mohammadi[1], Maryam Hemmatzadeh[2]

1. Alborz University of Medical Sciences, Karaj, Iran.
2. Tabriz University of Medical Sciences, Tabriz, Iran.

Background: Natural killer (NK) cells play a role in the pathogenesis of Ankylosing Spondylitis (AS) disease. Upregulated level of T-cell immunoglobulin and mucin domain 3 (TIM-3) is a sign of exhausted NK cells that might be regulated by microRNAs (miRNAs). Here in this investigation, we determined TIM-3 expression on NK cells (as a representation of NK cell exhaustion) in the AS patients and evaluated if miRNAs are involved in the modulation of TIM-3 expression in NK cells.

Methods: Peripheral blood specimens were obtained from 20 AS cases and 20 healthy subjects. NK cells were isolated by negative selection from a pool of Peripheral blood mononuclear cells (PBMCs). The frequency of TIM-3 expressing NK cells and expression of TIM-3 on NK cells were analyzed by flow cytometry. To measure expression levels of TIM-3 mRNA and miRNAs in the NK cells, Real-time PCR was exerted.

Results: The percentage of the TIM-3 expressing NK cells (p=0.032) and MFI of TIM-3 expression on the NK cells (p=0.015) were significantly increased in the AS cases compared to the controls. The mRNA expression of TIM-3 was significantly upregulated in the NK cells from AS cases compared to healthy cases (p=0.0014). The expression level of miR-155 (p=0.041) was significantly downregulated in the NK cells from AS patients in comparison to the healthy group.

Conclusions: Exhausted/functionally impaired NK cells are developed by upregulation of TIM-3 on NK cells (probably through miR-155) in AS patients.

Keywords: Ankylosing Spondylitis, T-cell immunoglobulin and mucin domain 3, Natural killer cell, miR-155.

Mutual Association between Autoimmune Diseases and Malignancies

Faezeh Shahba[1,2], Majid Khoshmirsafa[1,2], Farhad Seif[3]

1. Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran,
2. Department of Immunology, Iran University of Medical Sciences, Tehran, Iran
3. Department of Immunology and Allergy, Academic Center for Education, Culture, and Research, Tehran, Iran

Background: In recent decades, due to variations in the lifestyle of human societies, the prevalence of autoimmune diseases and cancers has increased significantly. AnImbalance in immune system responses is one of the key factors in this regard. There is a strong, dynamic, and mutual relationship between autoimmunity and cancer, although the underlying mechanisms of this relationship have not yet been elucidated. In some cases, therapeutic strategies used for the treatment of cancer and autoimmune diseases play a significant role in this relationship.
Methods: Genetics, epigenetics, inflammatory and anti-inflammatory cytokines, lymphocyte plasticity, disease mechanism, and host symbiotic microbiota play an important role in this correlation. Recent studies indicate that systemic autoimmune diseases are associated with a higher occurrence of cancers and malignancies. Chronic and systemic inflammation of these autoimmunities is one of the key justifications for this hypothesis.

Results: Constant stimulation of the immune system makes it ineffective and anergic, which favors the development of cancer. Currently, research has revealed that various cancer treatment approaches, such as immune checkpoint inhibitors (ICIs), monoclonal antibodies, and adoptive immunotherapy, lead to immune-related adverse events (irAEs) such as autoimmune diseases. One of the main reasons for these adverse events is the induction of immune responses against tumor tissue following immunotherapy, which causes tissue damage, loss of self-tolerance, and autoimmunity induction. These adverse events can occur in the tumor target organ or the other organs. It seems that there is an important correlation between the processes associated with the development of autoimmunity and cancer.

Conclusoin: According to limited information in this field, it is essential to increase studies to identify biomarkers, polymorphisms, genetic, and epigenetic predictors to assess this correlation and prevent the occurrence of autoimmune diseases and cancer simultaneously. In addition, immune-related adverse events can be predicted before drug administration to the patient by producing animal models and inducing both autoimmune disease and related malignancy.

Keywords: Autoimmune Disease, Cancer, Immune-related Adverse Events

Simultaneous Transduction of Dendritic Cells with A20 and BTLA Genes Stimulates the Development of Stable and Efficient Tolerogenic Dendritic Cells and Induces Regulatory T Cells

Mohammad Reza Javan[1]

1. Zabol University of medical sciences, Zabol, Iran.

Background: This study investigated the potential of simultaneous overexpression of A20 and B- and T-lymphocyte attenuator (BTLA) genes in dendritic cells (DCs) to develop a tolerogenic phenotype in DCs and investigate their capabilities for induction of immunosuppression.

Methods: Plasmid vectors were designed harboring A20, BTLA, and A20+BTLA genes and were transfected to HEK 293T cells to produce lentiviruses. DCs were transduced by the gene-carrying viruses and evaluated for the surface expression of MHCII, CD40, and CD86 molecules by flow cytometry. The mRNA expression of A20, BTLA, and CCR7 was determined. The mixed-lymphocyte reaction was conducted to evaluate the T cell stimulation potency and ELISA was used to measure the production of IL-10, TGF-β, and TNF-α. The potential of DCs for migration to lymph nodes and Treg induction were assessed by in vivo experiments.

Results: Transduction of DCs resulted in significantly decreased surface expression of CD40 and CD86 co-stimulators and upregulated A20, BTLA, and CCR7 mRNA expression. The IL-10 and TGF-β levels were enhanced significantly in the supernatant of LPS-treated DCs transduced with A20+BTLA-containing virus group relative to the DCs transduced with pCDH vectors. DCs transduced with the A20+BTLA harboring vectors had a higher migratory potential to mouse lymph nodes and caused the development of the higher numbers of Treg cells compared with the DCs transduced with pCDH vectors.

Conclusions: Simultaneous overexpression of A20 and BTLA genes in DCs caused the development of tolerogenic DCs with a promoted potential in the induction of Treg cells, accompanied by remarkable stability after inflammatory stimulation. All these offer promising potential for such DCs in treating autoimmune and inflammatory disorders.

Keywords: A20; BTLA, Immune tolerance, Tolerogenic dendritic cell, Autoimmunity

Study of FOXP3 Gene Polymorphisms in Systemic Lupus Erythematosus Female Patients with Skin Involvement and the Relation to Clinical and Paraclinical Characteristic

Paria Heydarinezhad[1], Nasser Gholijani[2], Zahra Habibagahi[3], Mohammad Reza Malekmakan[2], Zahra Amirghofran[1,4]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Autoimmune Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Internal Medicine, Rheumatology Division, Shiraz University of Medical Sciences, Shiraz, Iran
4. Autoimmune Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: FOXP3, is an important transcription factor of regulatory T cells and has shown an involvement in the development of various autoimmune diseases. To assess the association between FOXP3 gene polymorphisms (rs3761548 and rs2294021) on systemic lupus erythematosus (SLE) susceptibility and disease characteristics in female patients with skin involvement.

Method: Genotyping was performed on 108 female patients with SLE and malar rash and 133 female patients with SLE and photosensitivity separately and compared to 221 healthy female controls using PCR-RFLP. Patients' demographic, laboratory, and clinical information were all documented. The relationship between the SNPs and patients' characteristics was statistically analyzed.

Results: In female patients with photosensitivity, the frequency of CT genotype rs2294021 was significantly higher in comparison to healthy controls (OR=1.72; 95% CI=1.02-2.9, P=0.04). In both groups of patients with malar rash and photosensitivity, statistical analysis showed a correlation between FOXP3 SNPs with disease characteristics. In patients with malar rash, we found that the rs3761548 CC genotype was significantly higher in patients without abortion and gastrointestinal involvement. Decreased C4 level was more seen in patients with rs3761548 AC genotype. In patients with photosensitivity, we found a significant relationship between the rs3761548 AA genotype and a higher mean age of patients and also between the rs3761548 AC genotype and decreased C4 level and blood cell involvement. With respect to rs2294021 SNP, a significant association between CT genotype and higher mean ESR was observed.

Conclusion: FOXP3 rs2294021 CT genotype association with disease susceptibility in female patients with photosensitivity, and the correlation between FOXP3 SNPs and patient's characteristics and several clinical features may suggest the influence of FOXP3 on the disease development and pathology.

Keywords: Autoimmune Disease, FOXP3, Lupus, Polymorphisms, Regulatory T cell

Successful Treatment of the Bullous Disease of Pemphigus Vulgaris with the Widespread Mucocutaneous Lesions in an Old Patient

Mohsen Masjedi[1], Ali Asilian[1], Fazllolah Shariati[1], Banafshe Tamizifar[2], Fatemeh Andalib[1], Parvin Mahzoni[1], Vida Homayouni[1], Farzad Orayazi[3], Zabihollah Shahmoradi[1], Parvin Rajabi Dehnavi[1], Bahareh Abtahi Naeini[1]

1. Isfahan University of Medical Sciences, Isfahan, Iran.
2. Tehran University of Medical Sciences, Tehran, Iran.
3. Isfahan University of University of Medical Sciences of the Washington, D. C.

Background: Pemphigus vulgaris (PV) is a chronic and infrequent autoimmune mucocutaneous disease that is characterized by loose blisters and erosions on the skin and mucous membrane. Middle-aged adults are affected most frequently and the elderly and juvenile cases are infrequent. Herein, we reported a case of pemphigus vulgaris in an elderly patient.

Case Presentation: We reported a case of pemphigus vulgaris in an 85-year-old patient with widespread mucocutaneous lesions. We also reviewed the literature in MEDLINE with the keywords such as pemphigus vulgaris, elderly, mucocutaneous lesions, oral lesions, and treatment.

Conclusion: We have to stress that the importance of this case report is its presentation in an old patient, as the frequent age of presentation in Iranian patients is middle age. On the other hand, PV is a rare disease, thus, the reporting of any rare case with some exceptions is important.

Keywords: Cutaneous autoimmune diseases, Bullous Diseases, Pemphigus Vulgaris

The Effect of Conditioned Medium of Mesenchymal Stem Cells Treated with Nicotine on Concanavalin an Induced Autoimmune Hepatitis Induced in C57BL/6 Mice

Leila Mahmoudzadeh[1], Seyyed Meysam Abtahi Froushani[1], Rahim Hobbenaghi[1]

1. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Background: Autoimmune hepatitis is an autoimmune liver disease of unknown etiology. This disease seriously affects the lives of patients and if not treated, it leads to liver cirrhosis and irreversible liver failure. Con A is the best substance for inducing this disease in the experimental model of C57bl/6 mice. This substance causes the release of many cytokines and causes infiltration of active lymphocytes to the liver and liver damage. Mesenchymal stem cells of the cell population have a heterogeneous origin and are capable of self-renewal and re-differentiation. Nicotine also acts as an inhibitor of the immune system and has immune-modulating properties.

Methods: AIH models created by concanavalin A (ConA) and conditioned medium of MSCs with and without treated with nicotine injections at different times. The results of TGF-β, IL-10, and IDO assay were used to determine the optimal concentration of nicotine for in vivo tests. Finally, histopathology slides were prepared by H&E staining, and liver enzymes ALT, AST, and myeloperoxidase were calculated. The percentage of TCD4+ and TCD8+ lymphocytes in the blood was performed.

Results: A nicotine concentration of 0.5 micromolar was chosen to perform in ex vivo studies. Our experiments demonstrated that the levels of ALT, AST, MPO, and several inflammatory cytokines, including TNF-α, IFN-γ, and IL-6 were highest in the ConA group than in the other groups. Treatment with the conditioned medium of MSCs treated with nicotine caused a significant reduction. On the other hand, the same treatment increased the amount of IL-4 as an anti-inflammatory cytokine. Importantly, the numbers of activated TCD4+ and TCD8+ in the blood were calculated. These results showed that a conditioned medium of nicotine (with and without treatment with nicotine) makes the number of TCD4+ and TCD8+ cells decrease significantly.

Conclusion: The conditioned medium of mesenchymal stem cells, especially when treated with nicotine, can be used as a treatment for autoimmune hepatitis and rapidly assessing novel therapeutic approaches.

Keywords: Mesenchymal stem cells, Nicotine, Acute autoimmune hepatitis, ConA

The Expression Level of Immunological Checkpoints in Multiple Sclerosis Patients

Mohammad Javad Vaferi[1], Adib Miraki Feriz[1], Setareh Azarkar[1], Arezou Khosrojerdi[2], Hossein Safarpour[2]

1. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran
2. Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

Background: The most prevalent immune-mediated condition affecting the central nervous system is multiple sclerosis (MS). The largest global study to date estimates that there are 2.8 million people living with multiple sclerosis (MS) globally. The immune system is regulated by immunological checkpoints (ICs), which stop the immune system from randomly attacking its cells. However, by activating ICs targets, some malignancies can defend themselves against an onslaught. Studies in the past have revealed an increase in the number of ligands and receptors connected to ICs, which are important in the pathogenesis of autoimmune illnesses like multiple sclerosis. In this study, we used transcriptomics data to identify and assess the ICs ligands and receptors related to MS.

Methods: Starting, raw single-cell RNA sequencing data were downloaded using the code GSE138266 from the NCBI database for PBMCs of multiple sclerosis patients and normal donors. Data analysis was conducted by Python programming language using the Scanpy package. Following that, data was normalized and the effect of batches was removed. Finally, the expression pattern of ICs was constructed by dot plots. The Adjusted P-value for statistical analysis was computed using the Bonferroni procedure.

Results: According to the analysis that was done on PBMC, we found that CD28 and ICOS had significant increase expression in different clusters of T cells. Also, our investigations showed that in the population of activated B-cells that were in the patient sample, it has increased compared to the control sample, we see a high increase in CD40. Our more detailed data confirmed that there was increased expression of the C10orf54 gene in all cells examined, especially T-cell and monocyte populations.

Keywords: Multiple sclerosis, Immune Checkpoints, Autoimmune diseases, Immunotherapy

The Immunological Effects of Adipose Tissue Mesenchymal Stem Cells (AT-MSCs) in Females with Secondary Progressive Multiple Sclerosis: Clinical Trial Study

Fahime Lavi[1], Mahmoud Mahmoudi[1], Houshang Amiri2[2], Nafise Sadat Tabasi[1]

1. Department of Immunology and Allergy School of Medicine University of Mashhad, Mashhad Iran
2. Department of Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, the Netherlands

Background: Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) with pathological symptoms in affected people. Mesenchymal stem cells (MSCs) due to their immune-modulatory and neurogenerative effects have shown prospects in regenerative medicine. We have explored the safety, tolerability, and functionality of high doses of autologous adipose tissue MSCs (AT-MSCs) in 10 female patients with secondary progressive MS.

Methods: We screened for adverse events of AT-MSCs administration for 9 months following transplantation. Also, the immunomodulatory effect of MSCs was investigated by evaluating gene expression of inflammatory (IL1, IL6, IL17, IFN gamma) and anti-inflammatory (TGFβ, IL4, IL10, FOXP3) cytokines besides the proportion of peripheral blood T regulatory cells as important cells in autoimmune responses in MS diseases.

Results: Two injections of high doses of non-cryopreserved AT-MSCs were successfully administrated to 10 SPMS patients and no serious side-effect. The immunomodulatory effects of AT-MSCs were confirmed by enhancing the Tregs population and anti-inflammatory cytokines as well as lowering inflammatory cytokines in patients.

Conclusion: Administration of high-dose of non-cryopreserved autologous (AT-MSCs) was safe and well-tolerated in SPMS patients who participated in this study. This study was conducted with small sample size and grounds for more controlled trial studies to assess the longer-term safety and clinical efficacy of AT-MSCs transplantation in MS.

Keywords: Multiple sclerosis, Mesenchymal stem cells, T cells regulatory

Therapeutic Effect of Lactobacillus Plantarum on Lupus in Mice

Mohammad Ghasemi[1], Nakisa Sohrabi Haghdoost[2], Mobina Tahmasebizadeh[3], Zahra Akrami[3], Alireza Sadeghi nasab[4], Fatemeh Javadi[5]

1. Faculty of Nanotechnologynology, Institute of Royan, Isfahan, Iran
2. Department of Pathobiology, School of Veterinary Medicine, Science, and Research Branch, Islamic Azad University, Tehran, Iran
3. Students Research Committee, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
4. Department of Microbiology, Lahijan Branch, Islamic Azad University, Gilan, Iran
5. Department of Parasitology and Mycology, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Background: Lupus is a chronic autoimmune disease that can affect various organs in the body. It can lead to inflammation, pain, and damage to organs such as the skin, kidneys, and heart. A significant number of lupus patients suffer from gastrointestinal symptoms that can be attributed to a dysbiotic gut microbiome. Probiotics like Lactobacillus plantarum have been shown to have anti-inflammatory and immunomodulatory effects and could potentially benefit lupus patients.

Methods: An experimental study was conducted to investigate the efficacy of Lactobacillus plantarum in the treatment of lupus. Mice with induced lupus-like symptoms were orally administered Lactobacillus plantarum for 6 weeks. The mice were monitored for disease progression and tissue samples were taken for analysis.

Results: The study revealed that in comparison with a control group, the mice treated with Lactobacillus plantarum exhibited decreased systemic inflammation, reduced levels of autoantibodies, improved clinical symptoms, and alleviated renal and skin damage. The administration of Lactobacillus plantarum was also linked to the regulation of various immune cells, including T-helper cells, B-cells, and regulatory T-cells, which play a major role in the pathogenesis of lupus.

Conclusions: The results of this study suggest that the administration of Lactobacillus plantarum has a beneficial effect on lupus-like symptoms in mice. Thus, probiotics like Lactobacillus plantarum could potentially serve as a complementary or alternative therapy for lupus patients, particularly in those with gastrointestinal symptoms. However, further studies, particularly clinical trials, are required to confirm the therapeutic potential of probiotics in the treatment of lupus in humans.

Keywords: Lactobacillus plantarum, lupus

Umbilical Cord MSCs-Derived Exosomes Increased FOXP3+/ LAG-3+ Tregs and Ameliorates Experimental Autoimmune Encephalomyelitis

Adel Mohammadzadeh[1]

1. Department of Immunology and Genetic, Urmia University of Medical Sciences, Urmia, Iran

Background: The development of new strategies to control or alter the pathogenic CD4+ T cells during an autoimmune disease would be the major goal for the treatment of autoimmune disease.

Methods: Here we used Umbilical derived-mesenchymal stem cells (UMSCs)-exosomes (Exs) as an immune-modulatory agent to generate an attenuating immune response in experimental autoimmune encephalomyelitis (EAE).

Results: We showed that therapeutic administration of UMSCs-Exs increases the induction of Foxp3+/Lag3+ CD4+T cells in the spleen of EAE mice.

Conclusion: Given the functional similarities with their parental cells, MSCs, and their low safety concerns, UMSCs-Exs can be implicated as a safe Cell-free therapeutic approach in autoimmune diseases.

Keywords: EAE, UMSCs, Exosomes, Foxp3, Lag3, Tregs

Whole Exome Sequencing in A Consanguineous Iranian Family with Familial Multiple Sclerosis

Monireh Torabi Rahvar[1], Zahra Salehi[1], Farshid Noorakhsh[1], Maryam Izad[1]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Background: Multiple sclerosis (MS) is a complex autoimmune disease, with 20-50% of risk heritability attributable to common genetic variants. Recent studies have reported that the remaining heritability could be explained by low-frequency variants or mutations exclusive to multi-incident MS families. These variants could be enriched in populations with high rates of parental consanguinity. Thus, in this study, we aimed to identify possible rare variants in a consanguineous Iranian family with familial multiple sclerosis using whole exome sequencing.

Methods: A large multi-incident MS family with 4 affected members presenting Mendelian/near Mendelian form of MS was selected for this study. Whole exome sequencing was performed on four members of the family including two affected members who were first cousins and two non-affected parents with 4th degree of consanguinity. Exonic regions were enriched using an Agilent Sure Select Target V7-Post Enrichment Kit and paired-end sequencing was performed on the Illumina Nova Seq 6000 platform- with an average read length of 150 bases. We initially focused the analysis on rare variants with low population frequency (minor allele frequency below 0.01), then we prioritized variants that adhered to the segregation/zygosity pattern in the primary family (homozygous in MS patients and heterozygous in unaffected parents). Synonymous variants, and variants in intronic and intergenic regions were excluded from our analysis, then variants with predicted functional impact were selected and interpreted based on ACMG guidelines and literature review.

Results: We identified four distinct genomic variants in exonic regions of genes encoding for ZNF611, ANKRD36B, HLA-DRB5, and UBXN11. All the identified variants were consistent with a recessive autosomal inheritance pattern as suggested by the pedigree and were in a homozygous state in both affected members and presented as heterozygous regions in the two non-affected parents. Of these, two genes (ANKRD36B and HLA-DRB5) have not been previously reported as carrying MS-related rare variants. The variant identified in ANKRD36B was classified as possibly pathogenic using Franklin Genoox prediction tool.

Conclusion: Given that genetic susceptibility to MS has been linked to the HLA-DR15 haplotype (which encompasses HLA-DRB5*01:01 allele), the potential role of our newly identified variant in HLA-DRB5 deserves further investigation.

Keywords: Multiple Sclerosis, Familial MS, Consanguine Marriage, Whole Exome Sequencing, Rare Variant

Transplantation

Fabrication of Bioartificial Pancreas Using Decellularized Rat Testicular Tissue

Samaneh Karimi[1]

1. Abadan University of Medical Sciences, Abadan, Iran.

Background: Diabetes is a chronic disease that is associated with a decrease or disfunction of β-cell. In the present study, the fabrication of a bioartificial pancreas using a MIN-6 β-cell line seeded in decellularized rat testicles (testis-ECM) was investigated.

Methods: In this experimental study, the whole body of testes were decellularized and after characterization, were seeded by a MIN-6 cell line. The expression of insulin-related genes and proteins including Pdx-1, Glut2, Insulin, and Neurogenin-3 were evaluated. By using the radioimmunoassay method; insulin secretion was assessed under different concentrations of glucose. Seeded scaffolds with or without MIN-6 cells were transplanted to the rat's mesentery and their blood sugar and body weight were evaluated every three days for 28 days and analyzed with H&E staining. Histological assessments indicated the cells were completely removed after decellularization.

Results: The scaffold had no toxic impacts on the MIN-6 cells. Insulin release in response to different concentrations of glucose in 3D culture (testis-ECM) was significantly more than in the traditional 2D monolayer culture. Moreover, the relative genes and proteins expression were significantly higher in the 3D culture, compared to the 2D control group. In vivo, transplantation of the testis-ECM scaffolds showed appropriate positions for transplantation with angiogenesis and low infiltration of inflammatory cells. The recellularized scaffolds could drop blood sugar levels and increase the body weight of STZ-diabetic rats.III.

Conclusions: Our study clearly confirmed that ECM valuable organ scaffolds prepared by decellularization of the testicular tissue are suitable for the fabrication of bioartificial pancreas for transplantation.

Keywords: Diabetes, MIN-6 cells, decellularization, testis, scaffold

T Helper 17 and Regulatory T-Cell Profile and Graft-Versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation in Pediatric Patients with Beta-Thalassemia

Nima Rezaei[1], Shideh Namazi Bayegi[2], Amir Ali Hamidieh[3], Maryam Behfar[3], Amene Saghazadeh[4], Mahmood Bozorgmehr[5], Yalda Karamlou[3], Mehdi Shekarabi[6], Nader Tajik[6], Ali Akbar Delbandi[6], Farzaneh Tofighi Zavarh[7], Samaneh Delavari[4]

1. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Pediatric Cell and Gene Therapy Research Center, Gene, Cell and Tissue Research Institute, Tehran University of Medical Sciences, Tehran, Iran
4. Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
5. Oncopathology Research Center, Iran University of Medical Sciences, Tehran, Iran
6. Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
7. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment option for hereditary hemoglobin disorders, such as beta-thalassemia; however, this procedure is not without constraints, mainly engendering complications such as acute graft-versus-host disease (aGvHD), chronic GvHD (cGvHD), and susceptibility to infections.

Methods: The clinical outcomes of allo-HSCT are highly dependent on the quality and quantity of T-cell subsets reconstitution. Following the allo-HSCT of six pediatric patients afflicted with beta-thalassemia, their mononuclear cells were isolated, and then cultured with a combination of phorbol myristate acetate (PMA)/ionomycin and Brefeldin A.

Results: The content of CD4 T-cell subsets, including T helper 17 (Th17) cells and regulatory T cells (Tregs), were determined by specific conjugated-monoclonal antibodies three and six months post-HSCT. An increased frequency of total CD4 T-cells, Tregs, and Th17 cells was observed at day 90 and 180 after allo-HSCT, albeit the numbers were still lower than that of our healthy controls. In patients who developed cGvHD, a lower Th17/Treg ratio was observed, owing to a decreased proportion of Th17 cells.

Conclusion: The creating a balance between Th17 and Treg subsets may prevent acute and chronic GvHD in patients after allo-HSCT.

Keywords: Allogeneic hematopoietic stem cells transplantation, T-cell reconstitution, Regulatory T cell, T helper 17

TGF-B1, Smad3, Mir-21, and Mir-29 Genes Expression Patterns as the Possible Predictive Biomarkers of Interstitial Fibrosis and Tubular Atrophy during the First-Year Post-Renal Transplantation. A Cohort Report

Sanaz Keshavarz Shahbaz[1], Farshad Foroughi[2], Pedram Ahmadpoor[3], Ali Akbar Amirzargar[4]

1. Cellular and Molecular Research Center, Research Institute for Prevention of Non- Communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran
2. Department of Immunology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran.
3. Department of Nephrology, Shahid Labbafinejad Medical Center and Chronic Kidney Disease Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Department of Immunology, Faculty of Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran

Background: Interstitial fibrosis and tubular atrophy (IF/TA) are significant obstacles to successful kidney transplantation. This study aimed to evaluate the expression of pro-fibrotic (TGF-β1, Smad3, and miR-21) and anti-fibrotic (miR-29) genes during the first-year post-renal transplantation to suggest possible sensitive biomarkers for predicting IF/TA development.

Methods: Renal transplant recipients (n=52) were divided into well-functioning graft (WFG, n=27) and graft dysfunction (GD, n=25) based on the estimated glomerular filtration rate at 3rd-month post-transplantation. Blood samples and renal biopsies were obtained at baseline and 3, 6, and 12 months post-transplant. Gene expression analyses and pathological evaluations of the allografts were done using Taqman probe real-time PCR and the Banff classification, respectively.

Results: The expression levels of pro-fibrotic genes were significantly increased while the expression of the miR-29 gene was decreased in the peripheral blood of GD recipients in the 6th month (p<0.0001, for all comparisons). Similar results were found in the peripheral blood and allografts of recipients with abnormal pathological findings. Besides, the expression levels of the studied genes except for Smad3 could predict future interstitial fibrosis and tubular atrophy (IFTA).

Conclusions: Compared to the invasive biopsy-based methods, gene expression analysis of the peripheral blood of renal allograft recipients is a non-invasive technique that can introduce new genetic markers with enough sensitivity and specificity to predict the outcome of the transplant. Anyway, the obtained results need to be verified using larger sample sizes.

Keywords: Interstitial fibrosis and tubular atrophy (IF/TA), micro-RNA, renal transplantation, TGF-β1, Smad3

The Role of NK Cells and Their Exosomes in Graft Versus Host Disease and Graft versus Leukemia

Alireza Zafarani[1], Mahsa Taghavi-Farahabadi[2], Mohammad Hossein Razizadeh[3], Mohammad Reza Amirzargar[1], Mansoure Mansouri[4], Mohammad Mahmoudi[5]

1. Department of Hematology and Blood Banking, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
4. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Natural killer (NK) cells are one of the innate immune cells that play an important role in controlling tumors and viral diseases, but their role in hematopoietic stem cell transplantation is not yet fully understood. However, according to some research, these cells can prevent infections and tumor relapse without causing graft versus host disease (GVHD). In addition to NK cells, several studies are about the anti-leukemia effects of NK cell-derived exosomes that can highlight their roles in graft-versus leukemia (GVL).

Methods: PubMed and Google Scholar databases were searched for all available articles evaluating the role of NK cells and their exosomes in GVHD and GVL. Data from each study were collected individually and used for the writing of the article.

Results: There are both memory and training NK cells, so they learn through KIR receptors not to attack the host cells, and their toxicity is only for cells that do not express host MHCs in a small manner, which is the basis of the alloreactivity of NK cells. NK cells have been shown to be involved in a number of ways to reduce or increase GVHD. It can be said that regimen therapy and transplant donors will determine the role of NK cells in GVHD. NK cells reduce the chances of relapse after transplantation through the GVL effect, however, many factors such as therapeutic regimen and KIR-mismatch affect the potency of this effect. In addition, studies have shown that NK cell-derived exosomes have also anti-leukemia activities through some probable mechanisms such as cytotoxic proteins-mediated apoptosis, Fas/FasL-mediated apoptosis, and micro RNA-mediated inhibition of tumor cells.

Conclusion: It is expected that enhancing our knowledge in the field of NK cells results in improving transplantation outcomes and increasing the chances of successful treatment of infectious diseases and malignancies.

Keywords: Exosomes, GVHD, GVL, Hematopoietic stem cell transplantation, NK cell

Vaccine

A CRISPR-based live-attenuated Salmonella Typhi candidate vaccine can efficiently protect against the bacterium in mice

Yousof Tarverdizadeh[1], Mohammad Khalili[1], Saber Esmaeili[2], Gholamreza Ahmadian[3], Mehdi Golchin[1], Abbas Hajizade[4]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
2. Department of Epidemiology and Biostatics, Research Centre for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, Tehran, Iran
3. Department of Industrial and Environmental Biotechnology, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
4. Biology Research Center, Faculty of Basic Sciences, Imam Hossein University, Tehran, Iran

Background: Salmonella enteric serovar Typhi (S. Typhi) is the causative agent of typhoid fever, which annually affects 17 million people worldwide. The emergence of multidrug-resistant strains of S.Typhi bolds the necessity for the development of novel efficient vaccines against the bacterium. The present study evaluated the immunogenicity of a live attenuated bacterium, produced by the disruption of the gidA gene via the CRISPR/Cas9 system.

Methods: 107 CFU of the live attenuated stain was administered to BALB/c mice via the oral route, in a 3-dose regimen. Humoral, mucosal, and cellular immune responses were assessed. The protectivity of this candidate vaccine was evaluated using intraperitoneal administration of live virulent bacteria to control and immunize mice.

Results: The result showed that the candidate vaccine can stimulate the humoral immune system. Anti-capsular IgG in the sera of the immunized mice was significantly increased. The assessment of anti-capsular IgA antibodies in the fecal samples was also significantly increased in immunized mice, which indicates the proper stimulation of mucosal immunity. Indeed, the assessment of γ-interferon and interleukin-10 showed that the cellular immune system has been stimulated significantly. Finally, the challenge test showed that the immunized mice were able to tolerate at least 340 LD50 of the virulent bacteria.

Conclusion: The immunogenicity of the live attenuated vaccine candidate showed its efficiency in the immunization of mice against S. Typhi.

Keywords: CRISPR/Cas9, Live-attenuated vaccine, Salmonella Typhi, Cellular immunity, Protective immunity

A Nonpathogenic recombinant Leishmania tarentolae expressing sand fly PpSP15 and PsSP9 salivary proteins as a dual live vaccine candidate against Leishmania major and Leishmania tropica in BALB/c mice

Mahya Sadat Lajevardi[1], Tahereh Taheri[1], Elham Gholami[1], Hamzeh Sarvnaz[1], Sima Habibzadeh[1], Yousef Mortazavi[2], Negar Seyed[1], Sima Rafati[1]

1. Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran, Iran
2. Department of Medical Biotechnology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

Background: Leishmaniasis is a sand fly-transmitted parasitic disease threatening people worldwide. Although enormous efforts have been devoted to developing a promising vaccine, a human vaccine is not yet available. The protective potency of some specific sand fly salivary proteins has been already shown. Both PsSP15 from Phlebotomus papatasi and PsSP9 from Phlebotomus sergenti have considerable protection against L. major and L. tropica, respectively. This study aims to evaluate the protective potential of a dual saliva-based live vaccine using L. tarentolae expressing PpSP15 and PsSP9.

Methods: Here, recombinant L. tarentolae co-expressing, PpSP15, and PsSP9 were generated using the T2A peptide linker. Then, pre-treated BALB/c mice with CpG were immunized three times at three-week intervals subcutaneously at the footpad. Three weeks following the last immunization, the infectious challenge was carried out with L. major along with Ph. papatasi salivary gland homogenate or L. tropica plus Ph. sergenti salivary gland homogenate at the contralateral footpad of immunized mice. Different parameters were examined to evaluate the vaccine potency.

Results: The experimental evaluations of immune response demonstrated immunization with L. tarentolae co-expressing PpSP15 and PsSP9 potentially protected mice against both infectious agents in terms of reducing parasite load in draining lymph nodes and significant production level of IFN-γ versus the lower level of IL-10, IL-4, and IL-17.

Conclusion: Overall, the results indicated that the recombinant L. tarentolae expressing salivary proteins with CpG adjuvant can deviate immunity in favor of the Th1 response which is required to restrict Leishmania infection. Additionally, this dual live vaccine was introduced as a promising approach, especially in endemic areas where various species of sand flies transmit different Leishmania.

Keywords: Cutaneous leishmaniasis, Vaccine, Sand Fly, Salivary Proteins, Leishmania tarentolae

An in-house Human Influenza vaccine in MF-59 adjuvant: A report on vaccine potency and efficacy

Aref Sepasi[1], Mehri Ghafourian[1], Morteza Taghizadeh[2], Mehdi Mahdavi[3]

1. Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Department of Medical Vaccine, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
3. Recombinant Vaccine Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: The influenza a virus has been identified as a major global pandemic problem. Vaccination decreased the incidence in the communities, but many countries do have not access to enough doses of the vaccine, and should produce in-house. Here, we have reported the construction and potency of the recombinant H1N1 hemagglutinin vaccine as our in-house vaccine.

Methods: Recombinant H1N1 hemagglutinin was produced in the SF9 cell line and formulated in MF-59 adjuvant. Experimental mice were injected on days 0 and 14 with MF-59-formulated vaccine, commercial vaccine, and PBS. Cytokines IL-2, IL-4, and IFN-gamma were assessed with commercial ELISA. Antibody response by the hemagglutination inhibition and CTL activity by granzyme B ELISA was assessed. Moreover, the experimental challenge was performed to show the efficacy of the vaccine.

Results: A considerable rise in IFN-γ and IL-4 cytokine, as well as IFN-γ/IL-4 ratio, was seen versus the commercial vaccine and also the PBS group. Furthermore, our candidate vaccine showed superiority in humoral immune response and CTL activity versus the commercial vaccine and PBS group. The experimental challenge showed that the survival rate in the vaccinated groups revealed a significant increase versus the PBS group.

Conclusions: Our candidate vaccine showed a robust Th1 response and CTL activity versus the commercial vaccine. Moreover, a significant humoral immune response and higher survival rate were seen in our vaccine versus the commercial vaccine. It seems that the superiority of our vaccine is due to the type of vaccine formulation in the candidate vaccine.

Keywords: H1N1, Recombinant hemagglutinin, Vaccine formulation, IFN-γ, Immune response

Application of machine learning in the prediction of side effects of vaccines

Ehsan Ortogoli Farahani[1], Mehrnaz Mesdaghi[2]

1. School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Allergy and Clinical Immunology, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: It is critical to encourage vaccine administration as a way to reduce the impact of infectious diseases. Yet vaccine rates remain not sufficient because of negative attitudes and hesitancy, primarily due to concerns about side effects. On the other hand, Artificial intelligence (AI) has become helpful in many scientific fields. One of the fields in that AI caused great success is vaccinology. Predicting side effects is crucial when it comes to vaccines. Recent advances in supervised machine learning (ML) have gained much attention for predicting side effects because they can reduce time, design complexity, risk, and cost. A safer and more effective vaccine can be developed with the help of supervised ML techniques.

Methods: An electronic search was conducted in the PubMed database. The search strategy included the terms "Vaccine," "Machine learning," and "Side effects" in the title and abstract. The search was not limited to publications within a specific timeframe. The articles were examined concerning the selected topic to ascertain how machine learning assists in predicting vaccine side effects.

Results: Our search found 24 articles in PubMed. Twenty-one articles were excluded because they did not meet our inclusion criteria. On the whole, 3 articles were included.

Conclusion: AI and specifically ML can be considered as a robust tool in the prediction of vaccine side effects. The conventional assessment of vaccine side effects involves monitoring the effects of the vaccine on patients which is expensive and time-consuming. Thus, predicting the side effects with AI benefits investigators by reducing the cost and time of the experiments. However, this procedure is not widespread in the past years. But there is increasing research on recent COVID-19 vaccines and it seems that the utilization of AI in predicting vaccine side effects will grow in the future.

Keywords: Vaccines, Side effects, Machine Learning, Prediction

Characterization of a multi-epitope peptide with selective MHC-binding capabilities encapsulated in PLGA nanoparticles as a novel vaccine candidate against Toxoplasma gondii infection

Mona Roozbehani[1]

1. Iran University of medical sciences, Tehran, Iran.

Background: No effective human vaccine against Toxoplasma gondii (T. gondii) has yet been developed; however, a protective vaccine using immunogenic peptides in a safe delivery vehicle system offers promise.

Methods: Here, we employed bioinformatics to design a multimeric recombinant T. gondii vaccine using predicted T and B cell epitopes of SAG1, AMA1, ROP2, and GRA4 proteins based on their binding capabilities to common major histocompatibility complex (MHC) molecules. Furthermore, we encapsulated the expressed protein in poly lactic-co-glycolic acid (PLGA) nanoparticles as a delivery vehicle and also used alum as an adjuvant to determine the vaccine potency of this multimeric antigen. BALB/c mice were vaccinated and then challenged with the T. gondii RH strain, and the survival rate and cytokine profiles were studied.

Results: Mice vaccinated with the multi-epitope-based vaccine, both with and without PLGA, had greater Th1 immune responses, survival rates, specific antibody titers, and IFN-γ and IL-2 levels than controls, while the alum-adsorbed vaccine stimulated a Th2-type humoral immune response.

Conclusion:

Keywords: Toxoplasma gondii, Multi-epitope, PLGA nanoparticle, Vaccine

Combined alum and Glycyrrhetinic acid liposome against S. Typhimurium vaccine

Zahra zeinali[1], Seyed Meysam Abtahi Froushani[1], Abdolghffar Ownagh[1], Asghar Zamani[1]

1. Urmia University of Medical Sciences, Urmia, Iran

Background: Glycyrrhetinic acid (GA), an active principal aglycone of glycyrrhizin, has been shown to have immunostimulatory properties. This survey aimed to investigate the effects of a combination of alum and GA liposome on the induction of cellular and humoral immunity in response to a heat-killed preparation of Salmonella typhimurium (HKST).

Methods: Male BALB/c mice were immunized with the HKST alone or in combination with alum, GA liposome, or the alum-GA liposome mixture twice at a two-week interval. Fourteen days after the last challenge, immune responses against S. Typhimurium and the protective potential of the vaccines were monitored.

Results: The combination of alum and GA liposomes as an adjuvant enhanced the potential of the HKST challenge to augment lymphocyte proliferation, antibody titer, and delayed-type hypersensitivity reaction. These data were concurrent with the polarization of the immune response towards the Th1 response and the improvement of protective immunity against S. Typhimurium.

Conclusion: The combination of alum and GA liposome as adjuvant synergistically enhanced cellular and humoral immunity after immunization with the HKST vaccine.

Keywords: Glycyrrhetinic acid, Liposome, Salmonella typhimurium, vaccine, Adjuvant, Alum

Comparison of AstraZeneca and Sinopharm vaccines as boosters in protection against COVID-19 infection

Arash Letafati[1]

1. Tehran University of Medical Sciences, Tehran, Iran

Background: As the global number of confirmed cases rises past 640 million, vaccination remains the most effective measure in controlling COVID-19. Studies have shown that two doses of vaccination can significantly reduce hospitalization and mortality rates among patients, but the effectiveness of booster doses is also important. We aimed to evaluate the role played by the type of the 3rd dose of vaccination by comparing the safety and efficacy of two common vaccination histories differing only in the 3rd received dose.

Methods: We conducted a cross-sectional study on patients with respiratory symptoms suspected of having SARS-CoV-2 infection using Real-time PCR. We also collected information on the age, gender, and type of vaccine received for the third dose.

Results: Out of 346 cases with respiratory symptoms, 120 cases tested positive for SARS-CoV-2 and had received two doses of Sinopharm and a different booster dose of either AZD1222 (AstraZeneca) or BIBP (Sinopharm). Among these 120 patients, vaccination with AZD1222 as a booster dose resulted in fewer symptoms compared to those vaccinated with three doses of BIBP.

Conclusions: Our study demonstrates that booster doses can help reduce hospitalization and the severity of infection, and it appears that a combination of different vaccines may be effective against severe COVID-19 infection.

Keywords: AstraZeneca, Sinopharm, COVID-19, SARS-CoV-2

Designing an anti-breast cancer mRNA-based vaccine; Immunoinformatic AND immunologic logic

Marziyeh Ghayoumian[1], Fahimeh Shamsi[1], Hamid Madanchi[2], Mohammad Mehdi Ranjbar[3], Reza Jalalirad[4], Ramin Sarrami Forooshani[5], Mehdi Mahdavi[5]

1. Department of Biotechnology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran
2. Drug Design and Bioinformatics Unit, Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran Tehran Iran
3. Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
4. Production and Research Complex, Pasteur Institute of Iran, Karaj, Iran
5. Advanced Therapy Medicinal Product (ATMP) Department, Breast Cancer Research Center, Motamed Cancer Institute, Academic Center for Education, Culture, and Research (ACECR), Tehran, Iran. 6- Recombinant Vaccine Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: Active immunization in cancer therapy has various advantages, and the most important aspect is the induction of specific immune responses against tumors without toxicity for normal cells. Here, we aimed to develop a tumor vaccine based on mRNA technology. In fact, after the successful experience of SARS-CoV-2 vaccines developed by Pfizer and Moderna companies, this platform got more attention for various vaccine development.

Methods: The concurrent expression of several cancer antigens through an mRNA structure is possible. In addition, this type of vaccine is not limited to MHC, and also the low cost, fast, and easy production of vaccines in this platform, encouraged us to concentrate on the development of an mRNA vaccine against breast cancer encoding angiogenic antigens. Herein, VEGFR2 and c-MET as angiogenic elements were selected, and immunoinformatic analysis was performed for cytotoxic T lymphocyte activation.

Results: We also selected a few T helper epitopes as a helper agents for CTL response, given the role of Th in helping CTL. We also involved all in vivo and in vitro immunologically approved epitopes. Analyzes were performed using UniProt, IEDB, NCBI, and RCSB databases, BioEdit, MEGA, and Chimera tools.

Conclusion: Finally, we have selected some epitopes of these antigens and the project is ongoing to develop an mRNA vaccine.

Keywords: Breast cancer, mRNA vaccine, Immunoinformatic

Designing and in silico evaluation of a multi-epitope peptide-based vaccine against Monkeypox

Zahra Yazdani[1], Alireza Rafiei[2], Mohammadreza Yazdani[3], Amirreza Babaki[4]

1. Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran 2. Students Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
3. Department of Chemistry, Isfahan University of Technology, Isfahan, Iran
4. Students Research Committee, Mazandaran University of Medical Sciences, Sari, Iran.

Background: In 2022, Monkeypox becomes an outbreak in the world and has posed a serious threat to public health worldwide. The serious bioinformatics approaches were implemented to design an efficient multi-epitope vaccine against it.

Methods: Immunodominant epitopes from B21R, A33R, J3R, D3R, and B19R proteins were selected based on the stimulation of humoral and cellular immunity, antigenicity ability, and allergenicity probability. The appropriate adjuvants in the vaccine structure were inserted to potentiate the immunogenicity of the antigens. The vaccine segments were connected by appropriate linkers. The physicochemical properties, structural stability, and immunological characterizations of the vaccine were evaluated. Modeling, refinement, and validation were performed to access a high-quality three-dimensional structure of the vaccine protein. Docking and molecular dynamics simulations were performed between the vaccine and toll-like receptors (TLRs) 3, 4, and 8. In silico cloning and mRNA stability were predicted. Prediction of immune stimulation after injection in the human body was done.

Results: The vaccine contains 613 amino acids constructed from peptides from antigens and virulence factors B21R, A33R, J3R, D3R, and B19R. The vaccine might induce humoral and cellular immune responses against MPXV. Also, it has a high-quality structure and suitable physicochemical properties. Docking and molecular dynamics simulation of the vaccine with TLR 3, 4, and 8 showed an appropriate and stable interaction between the vaccine and immune receptors. In silico cloning showed the vaccine protein may transcript and translate in Escherichia coli.

Conclusion: Totally, a potential vaccine candidate with proper immunological and stable physicochemical properties against Monkeypox was designed. It is expected the vaccine could be capable to protect humans from Monkeypox.

Keywords: Monkeypox, Multi-epitope peptide-based vaccine, Cellular immunity, Humoral immunity

Designing of Multi-Epitope Vaccine against Trichomonas vaginalis Using Immunoinformatic

Babak Beikzadeh[1]

1. Department of Cell and Molecular Biology & Microbiology, Faculty of Biological Sciences and Technology, University of Isfahan, Isfahan, Iran

Background: Trichomonas vaginalis is one of the most common sexually transmitted infections in the world, which is associated with complications of infertility, risk of other sexually transmitted diseases, risk of cervical cancer, low birth weight infants, and HIV transmission. Given the importance of this infection in public health, extensive efforts have been made to develop vaccines. Previous research has been limited to the killed vaccine or using an adhesion protein as a vaccine candidate, and no effective vaccine for the disease has been suggested until now. This study aimed to design a vaccine based on epitopes of parasite adhesion proteins as an immunogenic protein using immunoinformatic tools.

Methods: First, AP33, AP51, and AP65 protein sequences were retrieved. Epitopes of B and T lymphocytes were then predicted. Antigenicity, non-allergenicity, and non-toxicity of epitopes were evaluated and vaccine structure was designed. Then the physical, chemical, and structural properties of the vaccine were determined and finally, the ability of the vaccine to bind to TLRs was investigated.

Results: A total of 9 lymphocytes B and T epitopes were selected and a vaccine construct was designed based on them. Immunoinformatic evaluations showed that the designed vaccine is safe, hydrophilic, and stable at different temperatures and conditions that can bind to TLRs and activate innate immunity.

Conclusion: Based on the results, the polypeptide construct can be a suitable candidate for Trichomoniasis vaccine.

Keywords: Trichomonas vaginalis, Vaccine, Adhesion proteins, Immunoinformatic

Enhancing antibody responses to Plasmodium falciparum generative cell-specific 1 antigen, designed as self-assembled peptide nanoparticles (SAPN)

Anita Lotfi[1], Leila Nourani[1], Hediye Vand-Rajabpour[1], Akram Abouie Mehrizi*[1]

1-Malaria and Vector Research Group (MVRG), Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran

Background: Malaria is one of the most important infectious diseases in the world. In addition to the usual control methods, the development of new tools such as vaccines is required to eliminate and eradicate malaria. Among vaccines, nano vaccines such as self-assembled peptide nanoparticles (SAPNs) are suitable carriers for vaccines due to their particulate repetitive antigen display characteristics that get together spontaneously in the dialysis processes. SAPNs can efficiently stimulate the immune system and increase the responses related to recombinant antigens. One of the identified transmission-blocking vaccine (TBV) candidate antigens is Generative Cell-Specific and has been designed as SAPN in our laboratory. In the current study, enhancing the immune responses to the SAPN form of PfGCS1 in comparison to recombinant PfGCS1 was evaluated.

Methods: After protein expression and purification of antigens, purified PfGCS1-SAPN dialyzed in a gentle concentration gradient of urea. Then, the size and shape of the obtained SAPN were determined by Dynamic Light Scattering (DLS) and Field Emission Scanning Electron Microscopy (FESEM) tests, respectively. Then, mice were immunized subcutaneously 3 times in 2 weeks intervals in 8 distinct groups. The level and titer of IgG antibody responses to target antigens were evaluated using ELISA, after the third immunization.

Results: The size of PfGCS1-SAPN was determined between 25-60 nm and FEMES confirmed the spherical shape of PfGCS1-SAPN. Evaluating the immune responses in the mouse groups immunized with PfGCS1-SAPN showed a higher level and titer of IgG antibody responses relative to mice receiving recombinant PfGCS1.

Conclusion: The results of this study revealed the strong immunogenicity of the nano form of antigen (PfGCS1-SAPN), suggesting that this platform can be applied for designing vaccines against malaria.

Keywords: Self-assembled peptide nanoparticle (SAPN), Malaria vaccine, PfGCS1, Nanoparticles

Expression and production of the toxin-coregulated pili A (TCPA) of Vibrio cholera and investigating its detection and neutralization power

Vahid Hayati[1], Abbas Hajizade[1], Shahram Nazarian[1], Yousof Tarverdizadeh[1], Keivan Nasirzadeh[1], Mohammad Reza Akbari[1]

1. Biology Research Center, Faculty of the Basic Sciences, Imam Hossein University, Tehran, Iran

Background: Vibrio cholerae (V. cholera) is responsible for the life-threatening disease, cholera that annually infects 1.3 to 4 million people worldwide of which 21,000 to 143,000 patients die. Indeed, over the past decades, cholera outbreaks have been reported in more countries. On-time diagnosis as well as efficient preventive strategies, such as vaccination, play an important role in the reduction of the disease burden and mortality. In this regard, in the present study the raised antibodies against a virulence factor of the bacteria, called toxin-coregulated pili A (TCPA), in the detection and neutralization of V. cholerae, was investigated.

Methods: For this aim, the tcpA gene was cloned in the pET32a (+) expression vector and expressed in the E. coli expression vector. The protein expression was analyzed by SDS-PAGE and Western blotting and purified using a Ni-NTA column. To investigate if the raised antibodies against TCPA can detect and neutralize V. cholerae, purified TCPA was subcutaneously administered to BALB/c mice and the sera of the immunized mice were obtained. The efficiency of the raised antibodies for the detection of V. cholerae was assessed using ELISA. A suckling mouse assay was used for the investigation of the neutralization power of the antibodies.

Results: The result of the expression study showed that the protein has been successfully expressed and purified. ELISA showed that the raised antibodies can detect whole bacterial cells. Indeed, oral administration of the active V. cholerae to suckling mice showed the efficiency of TCPA-specific antibodies in the neutralization of the bacterial cells.

Conclusion: In conclusion, the present study showed the efficiency of TCPA antibodies in the detection and neutralization of V. cholerae; so, it can be used for the development of a diagnostic kit for the bacterium. Indeed, it can be used as an important part of vaccines against the bacterium.

Keywords: Vibrio cholera, Cholera, TCPA, Diagnostic Kit, Vaccine Candidate

Frequency of B-cell subpopulations in low responders in comparison with high responders to hepatitis B vaccine among health care workers

Zahra Saleh[1], Fereshteh Mehdipour[2], Kourosh Kalantar[2]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Prevention of hepatitis B is the most effective control measure. Vaccination is the best way to prevent this infection. Immunological memory constitutes the basis of vaccination. Hence, determining the frequency of memory B-cell (MBC) subsets is an important indicator of vaccine efficacy. This study aimed to evaluate the frequency of different B-cell subpopulations and the expression of PD-1 on B-cell subsets in low responders compared to high responders of hepatitis B vaccinated healthcare workers.

Methods: The peripheral blood of 41 healthy vaccinated individuals was collected who were classified into two groups based on the Anti-HBsAb titer, 20 LR, and 21 HR. Flow cytometry analysis was used to determine the frequency of B cell subpopulations and PD-1 expression level on B cell subsets and enzyme-linked immunosorbent assay (ELISA) for Anti-HBsAb titer.

Results: No significant differences were found in the frequency of various B-cell subpopulations and the expression level of PD-1 on B-cell subsets between LR and HR groups. The expression level of PD-1 was significantly higher on atypical MBC (atMBC) than that of naive B cell ($p<0.001$) and classical MBC (cMBC) ($p<0.05$) in LR and HR groups. Moreover, cMBCs had a significantly higher PD-1 expression than naive B cells in the LR group ($p=0.0158$). We observed a negative correlation between age and HBsAb titer ($p=0.0011$, r: -0.6751), a positive correlation between age and PD-1 expression level on cMBC ($p=0.0458$, r: 0.4513), a negative correlation between HBsAb titer and immature B-cell frequency ($p=0.0347$, r: -0.4740) and a positive correlation between HBsAb titer and cMBC frequency ($p=0.0223$, r: 0.5077) in the LR group.

Conclusion: Low responsive rate to the HB vaccine in healthy individuals may be due to the defect in the Specific immunological memory, rather than a global immune dysfunction.

Keywords: Hepatitis B vaccine, Memory B cell, PD-1

Immune Response to Measles Virus in Iranian vaccinated Young Adults

Maryam Keshavarz[1], Sina Soleimani[2], Maryam Izad[3], Majid Tebyanian[4], Soheyla Moradi[4], Gholamreza Tariqi[4], Ahmad Yousefi[4]

1. Department of quality control, Razi Vaccine and Serum Research Institute
2. Department of quality control, Razi Vaccine and Serum Research Institute
3. Department of Immunology, Medical School of Tehran University of Medical Sciences
4. Razi vaccine and Serum Research Institute

Background: Measles is a highly contagious viral disease (RO =12-18) that can be prevented by vaccination. The gold standard for evaluating the effectiveness of vaccines is the antibody titer, while the avidity of the antibody and the cellular immune response are very important in this regard.

Methods: In this study, the humoral and cellular immune responses were performed in 20-30-year-old adults who had received 2 or 3 doses of live attenuated measles vaccine as children. ELISA was used to evaluate humoral immunity and flow cytometry was used to evaluate cellular immunity.

Results: The average measles-specific IgG titer in the age group of 23-20 years old was higher than the age group of 27-24 years old (p=0.56) and the age group of 28-30 years old (p=0.24). The average value of measles-specific IgG avidity (p<0.01) in the studied population was significantly different from the positive control. In this study, 14 people (15.5%) who were seropositive for measles did not have specific IgG with high avidity. No significant difference was observed between the mean measles-specific IgG avidity between people who received two or three doses of measles vaccine (p=0.57). The mean percentage of specific TH1 cells that produce IFNγ in response to the measles virus is significantly (p<0.01) higher than the mean percentage of virus-specific TH2 cells. The mean frequency of IFNγ+ CD8+ T cells specific for the measles virus (p>0.01) was significantly higher than the mean frequency of CD107a+ CD8+ T cells. The proliferative response of measles-specific CD8+ T and CD4+ T lymphocytes in 100% of seropositive individuals and the serum was negative. The proliferative response in CD8+ T lymphocytes was higher than in measles-specific CD4+ T lymphocytes (p=0.3), but this difference was not significant. The amount of IL12p40 in the mononuclear cell culture medium in response to measles viruses was insignificant.

Conclusion: Considering that IgG avidity was low in some seropositive people, as well as the presence of cellular immune response in seronegative people, it can be concluded that the specific antibody titer is not enough to check the effectiveness of vaccines.

Keywords: Measles, Vaccination, Immune response

Immunity against rubella virus in vaccinated young Iranians

Maryam Keshavarz[1], Maryam Izad[2], Iraj Khalili[1], Gholamreza Tarighi[1], Ahmad Yousefi[1]

1. Razi Vaccine and Serum Research Institute
2. Tehran University of Medical Sciences, Department of Immunology, Tehran, Iran

Background: Rubella is a contagious viral disease. Most people who get rubella usually have a mild illness. Rubella infection in pregnant mothers can cause congenital rubella syndrome in babies. Although antibody titer is the gold standard, antibody avidity and cellular immune response are important in assessing immune status.

Methods: In this study, the humoral and cellular immune responses were performed in 20-30-year-old adults who had received 2 or 3 doses of MMR vaccine as children. ELISA was used to evaluate humoral immunity and flow cytometry was used to evaluate cellular immunity.

Results: In this study, 100% of rubella seropositive people had specific IgG with high avidity. The frequency of specific TH1 cells that produce IFNγ in response to rubella viruses is significantly (p<0.01) higher than the average frequency of virus-specific TH2 cells. The mean frequency of IFNγ+ CD8+ T cells specific for the rubella virus (p>0.05) was significantly higher than the mean frequency of CD107a+ CD8+ T cells. There is no significant relationship between the component of the humoral immune response and the cellular immune response to, the rubella virus.

Conclusion: The immune status of Iranian vaccinated young adults against rubella has remained stable over the years.

Keywords: Rubella, Vaccine, Immunity

Immunogenicity and protective efficacy of a newly developed acellular Pertussis vaccine in a murine intranasal model

Danyal Imani[1], Tannaz Bahadori[1], Sedigheh Ghourchian[2], Forough Golsaz Shirazi[3], Masoumeh Douraghi[4], Mahmood Jeddi Tehrani[5], Mohammad Mehdi Amiri[6], Fazel Shokri[6]

1. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4. Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
5. Monoclonal Antibody Research Center, Avicenna Research Institute, Academic Center for Education, Culture, and Research (ACECR), Tehran, Iran
6 Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Background: Pertussis is a vaccine-preventable disease caused by the gram-negative bacterium Bordetella pertussis (B. pertussis). Acellular pertussis vaccines (aPV) which are composed of three or more immunogenic components of B. pertussis display similar efficacy, but less reactogenicity as compared to the whole-cell pertussis vaccines (wPV). We developed an aPV vaccine composed of native filamentous hemagglutinin (FHA), pertactin (PRN), and pertussis toxin (PT) subunits emulsified with diphtheria and tetanus toxoids (DT) and evaluated its immunogenicity and protectivity in a murine model.

Methods: Three immunogenic components of B. pertussis including FHA, PRN, and PT were extracted and purified from supernatant and pellet of bacterial culture. BALB/c female mice were used for the assessment of immunogenicity and protective efficacy. Two doses of acellular pertussis vaccine were administered intraperitoneally (IP) at 3-week intervals. According to the type of vaccine, five groups of mice were allocated. Two commercial vaccines (Infanrix and Boostrix) composed of the same subunit antigens with different concentrations served as positive controls (groups 1 and 2). Two formulations of our vaccine with the same concentrations of the subunit antigens as the commercial vaccines were also included (groups 3 and 4). The fifth group received the DT component as a control. Anti-PT, anti-FHA, and anti-PRN antibody titers were measured after primary and booster vaccinations by ELISA. An intranasal challenge test was also performed 2 weeks after booster vaccination to determine protective efficacy by assessment of pertussis colony forming unit (CFU) in lungs, 2 hours and 10 days after the challenge.

Results: A significant increase in antibody titers against all pertussis antigens were detected after the first and booster dose vaccinations in the serum of all groups immunized with pertussis vaccines compared to the control group. No significant differences were observed between the groups receiving the commercial vaccines compared to our vaccines. The CFU results obtained after the intranasal B. pertussis challenge revealed complete eradication of infection 10 days after bacterial challenge in all pertussis-immunized groups as opposed to the control group.

Conclusion: Our pertussis vaccine formulation which is the first aPV candidate vaccine developed in Iran displays immunogenicity and protective efficacy similar to the standard commercial vaccines and deserves further investigation in humans.

Keywords: Pertussis, Acellular pertussis vaccine, Immunogenicity, Bacterial challenge, Animal model

Immunogenicity and safety of the BBIBP-CorV vaccine in patients with autoimmune inflammatory rheumatic diseases undergoing immunosuppressive therapy in a monocentric cohort

Hossein Motedayyen[1], Batool Zamani[1], Ahmad Piroozmand[1]

1. Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran

Background: Vaccination plays a fundamental role in mastering the COVID-19 pandemic and protecting vulnerable groups. Persons with autoimmune inflammatory rheumatic diseases (AIIRD) requiring immunosuppressive therapies are prioritized for vaccination. However, data concerning the immunogenicity and safety of the BBIBP-CorV vaccine in immunosuppressed patients are not found. This study presents data on the efficacy and safety of the BBIBP-CorV vaccine in immunosuppressed patients compared to healthy controls.

Methods: The study population consisted of 100 healthy controls and 100 patients with AIIRD. Vaccination was performed according to national guidelines with the BBIBP-CorV vaccine. SARS-CoV-2 neutralizing antibody titers were quantified by ELISA before initial vaccination and 1–3 months after secondary vaccination. Adverse events were assessed before study initiation and 7 days after the second dose. Disease activity was studied before entering the study and 3-8 weeks after the second dose.

Results: Vaccination-induced positive immunogenic response rates and SARS-CoV-2 neutralizing antibody titers were significantly lower in the AIIRD patients than in healthy subjects (p<0.05). There are significant differences in neutralizing antibody titers among patients suffering from RA, SLE, SSc, and AS (p<0.01-0.05). The rates of seropositive vaccine responses were similarly distributed across all diseases. Healthy and AIIRD individuals had a similar profile in adverse events. No significant difference was observed in SARS-CoV-2 antibody titers between subjects suffering from side effects and those who did not have them. SARS-CoV-2 neutralizing antibody levels were significantly higher in subjects with a history of COVID-19 infection than in seronegative individuals (p<0.01-0.05). Seropositive subjects had a significant increase in the percentage of vaccine-related adverse events compared to seronegative persons (p<0.05). Despite a minor change in the disease activity of patients with RA and SLE, disease activity indices were overall stable in the AIIRD patients.

Conclusion: These findings revealed that the BBIBP-CorV vaccine is effective in the development of neutralizing antibodies in immunosuppressed patients without considerable reactogenicity or induction of disease flares.

Keywords: Immunogenicity, Adverse events, Disease activity, BBIBP-CorV vaccine, Patients with AIIRD

Immunogenicity of Anopheles stephensi alanyl aminopeptidase N1 antigen formulated with CPG, MPL, or QS-21 adjuvants as a Malaria transmission-blocking vaccine candidate

Zeinab Pourhashem[1], Akram Abouie Mehrizi[1], Leila Nourani[1], Sakineh Pirahmadi[1], Abbasali Raz[1], Sedigheh Zakeri[1], Navid Dinparast Djadid[1]

1. Malaria and Vector Research Group, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

Background: Malaria is a significant infectious disease transmitted by an infected mosquito biting humans. According to the latest WHO report, about 627000 people died in 2020, and malaria is still one of the leading causes of death in endemic regions. Considering the goal of the elimination and eradication program, which is to reduce the transmission of Plasmodium, transmission-blocking vaccines (TBV) have received more attention. Among the promising TBV candidates, Anopheles stephensi alanyl aminopeptidase N (AnAPN1), a ligand for Plasmodium ookinetes, has shown the potency to interrupt the life cycle of Plasmodium species in anopheles' midgut. This study was designed to evaluate the immunogenicity of AnAPN1 formulated with CpG, MPL, or QS-21 adjuvants in BALB/c mice.

Methods: BALB/c mice were immunized three times in two weeks intervals with recombinant APN1 alone or with each of CpG, MPL, and QS-21 adjuvants. Assessment of humoral and cellular immune responses in immunized mouse groups was evaluated using conventional enzyme-linked immunosorbent assay (ELISA) to investigate IgG antibody titer, isotype profiling, and avidity in the post-immunized mice after the second boost.

Results: Evaluation of the humoral responses in vaccinated mice manifested a significant increase in the high-avidity level of anti-APN1 IgG and IgG subclasses in mice receiving rAPN1 formulated with adjuvants. Furthermore, rAPN1 induced the highest amount of anti-APN1 IgG2a and IgG2b antibodies (as Th1 response index) in mice receiving APN1-QS-21 formulation.

Conclusion: The results have shown that APN1 formulated with QS-21 can stimulate high affinity with higher titer anti-APN1 antibodies versus the mice that received antigen alone. The obtained results showed that APN1-QS21 is the best among examined formulations and encouraged us to perform the transmission-blocking activity of this formulation in upcoming projects.

Keywords: Malaria, AnAPN1, Transmission-blocking vaccines (TBV), Adjuvant

Immunoinformatics design of multi-epitope vaccine using OmpA, OmpD, and enterotoxin against non-typhoidal salmonellosis

Babak Beikzadeh[1]

1. Department of Cell and Molecular Biology & Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran

Background: Non-typhoidal Salmonella (NTS) is one of the important bacteria that cause foodborne diseases and invasive infections in children and elderly people. Since NTS infection is difficult to control due to the emergence of antibiotic-resistant species and its adverse effects on the immune response, the development of a vaccine against NTS would be necessary.

Methods: This study aimed to develop a multi-epitope vaccine against the most prevalent serovars of NTS (Salmonella Typhimurium, Salmonella Enteritidis) using an immunoinformatics approach and targeting OmpA, OmpD, and enterotoxin (Stn).

Results: Initially, B cell and T cell epitopes were predicted. Then, the epitopes and suitable adjuvants were assembled by molecular linkers to construct a multi-epitope vaccine. Computational tools predicted the tertiary structure, refined the tertiary structure, and validated the final vaccine construct. The effectiveness of the vaccine was evaluated via molecular docking, molecular dynamics simulation, and in silico immune stimulation. The vaccine model showed good binding affinity and stability with MHC-I, MHC-II, and toll-like receptors (TLR-1, 2, 4), as well as activation of T cells, IgM, IgG, IFN-γ, and IL-2 responses. Furthermore, after codon optimization of the vaccine sequence, this sequence was cloned in an E. coli plasmid vector pET-30a (+) within the restriction sites of HindIII and BamHI.

Conclusion: This study, for the first time, introduced a multi-epitope vaccine based on OmpA, OmpD, and enterotoxin (Stn) of NTS that could stimulate T and B cell immune responses and be produced in the prokaryotic system. This vaccine was validated in the in-silico phase, which is an essential study to reduce challenges before in vitro and in vivo studies.

Keywords: Non-typhoidal Salmonella, OmpA, OmpD, Enterotoxin, Immunoinformatics, Multi-epitope vaccine

In silico analysis of multi-epitope peptide vaccine candidate against human brucellosis

Zahra Yazdani[1], Alireza Rafiei[1], Mehrafarin Ghoreyshi[2], Saeid Abediankenari[3]

1. Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Students Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
3. Immunogenetics Research Center, Department of Immunology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Background: Brucellosis is one of the neglected endemic zoonoses in the world. Vaccination may be a promising health strategy to prevent this disease. This study applied advanced computational techniques to develop a potent multi-epitope vaccine for human brucellosis.

Methods: The protein antigens of the B. melitensis strain were accessed by electronic databases of Google Scholar, PubMed, EMBASE, Scopus, and Web of Science and retrieved from the NCBI databases. Immunogenic epitopes from the antigens that had the potential for cellular and humoral responses and didn't have allergenic probability were selected. Suitable adjuvants were added to the vaccine structure to improve its immunogenicity. The physicochemical and immunological properties of the vaccine were evaluated. The two and three-dimensional structure was predicted. The vaccine was docked with toll-like receptor 4 for stimulating innate immune responses. In silico cloning, codon optimization, and mRNA stability evaluation were performed to evaluate the vaccine protein expression in Escherichia coli. The immune simulation was done to reveal the immune response profile of the vaccine after injection.

Results: Seven epitopes from four main species of Brucella which infect humans were selected. These epitopes and Heparin Binding Hemagglutinin, PADRE, and the epitopes identified from the Tetanus toxin fragment C were joined to produce a vaccine structure. The vaccine has a high-quality structure and immunological and physicochemical properties. It has appropriate interaction with TLR4. It expresses in E. coli efficiently. The immune simulation showed that the vaccine induces cellular and humoral immune responses in the host body.

Conclusion: The vaccine may have the ability to induce the immune response, especially cellular responses to human brucellosis. This vaccine has appropriate physicochemical properties, a high-quality structure, and a high potential for expression in a prokaryotic system. However, these results need to be verified by experimental methods.

Keywords: Human brucellosis, multi-epitope peptide vaccine, *in silico*, Immune stimulation, Toll-like-receptor 4

In silico studies for selecting the B- and T-cell epitopes of BP26 protein from Brucella melitensis as a vaccine candidate

Kimia Talebi[1], Mehri Habibi[2], Morteza Samadi[3], Mohammad Reza Asadi Karam[2]

1. Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Molecular Biology, Pasteur Institute of Iran, Tehran, Iran
3. Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Background: Brucellosis is one of the major zoonosis diseases around the world and is recognized as an endemic disease in Iran. Despite the use of various strategies in the design of vaccines such as lived vaccines, killed vaccines, and subunit vaccines, there is still no effective human vaccine against Brucella. The newly investigated methods are based on epitope vaccines. BP26 is a major protein of Brucella melitensis. Thus, in this study, we aimed to select the best B- and T-cell epitopes of BP26 protein as an epitope-based vaccine.

Methods: The BP26 protein sequence was obtained from the Gene Bank database. B- and T-cell epitopes of the protein were predicted using different software programs like ABCpred and BepiPred for B-cell prediction and IEDB for T-cell prediction. Final B- and T-cell predicted epitopes were evaluated using the VaxiJen 2.0 server.

Results: The best B-cell epitopes were selected according to the criteria based on cutoff values for ABCpred and BepiPred which was >0.8. Finally, three B-cell epitopes were predicted. For the prediction of T-cell epitopes, two alleles DRB10101 and HLA A0201 as common alleles for MHC-II and MHC-I in humans were used. For MHC-II, three epitopes and for MHC-I, two epitopes with the highest scores were selected. The antigenicity of these regions was identified by the VaxiJen 2.0 with a score >0.4.

Conclusion: The advancement of immunoinformatic servers and the huge disclosure of protein data have an important role in the development of epitope-based vaccines. An epitope that can produce both B-cell and T-cell mediated immunity is highly desirable for designing epitope-based vaccines. The objective of the study was to find the Tcell and Bcell epitopes of BP26 protein for vaccine design against Brucella melitensis. To prove the effectiveness of the epitopebased vaccine, in vivo and in vitro studies are under investigation.

Keywords: Brucella melitensis, BP26, Epitopes, Vaccine

Influenza vaccine booster stimulates antibody response in beta-thalassemia major patients

Rasoul Baharlou[1], Abbas Ahmadi Vasmehjani[2], Maryam Sheikh[2]

1. Department of Immunology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
2. Department of Immunology and Microbiology, School of Medicine, Jahrom University of Medical Sciences, Jahrom, Iran

Background: The present study aimed to evaluate antibody response against the influenza vaccine in beta-thalassemia major patients from Iran.

Methods: Thirty beta-thalassemia major patients were enrolled and divided into three groups, namely single dose (group 1), double dose (group 2), and control (group 3). Seroconversion, seroprotection, and geometric mean titer (GMT) assays were performed through hemagglutination inhibition (HI) on days 0, 14, and 60.

Results: Based on the results, the level of antibody titer was increased in group 2. Two weeks after vaccination, the seroconversion rate was about 20% and 30% in groups 1 and 2. Sixty days after vaccination, the seroconversion rate was around 70% and GMT showed a more than 2-fold increase in group 2.

Conclusion: Based on the results, the immunogenicity of double dose vaccination against influenza infection appears to be higher than the single dose vaccine in beta-thalassemia major patients and thus, it is recommended to use two doses of vaccine, especially in splenectomized patients who are more sensitive than others.

Keywords: Influenza virus vaccination, Beta-thalassemia major, Seroconversion, Seroprotection, Geometric mean titers

Investigation of frequencies of various B cell populations in non-responder health care workers in comparison to responders to HBV vaccination

Sara Karimi[1], Fereshteh Mehdipour[2], Jamal Sarvari[3], Kourosh Kalantar[4]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences
3. Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Hepatitis B infection is one of the most common infectious diseases in the world. More than 90% of hepatitis B vaccinated immunocompetent adults are fully immunized. The main goal of vaccination is to produce humoral memory. Having a lower percentage of total or antigen-specific memory B cells in non-responders in comparison to responders is still a controversial issue. The study aimed to investigate the frequency of various B cell subpopulations in non-responders compared to responders.

Methods: 14 responders and 14 non-responders of Shiraz Hospital healthcare workers enrolled in this study. Flow cytometry was used for the evaluation of various CD19+ B cell subpopulations using fluorescent-labeled anti-CD19, -CD10, -CD21, –CD27, and -IgM.

Results: We found that there were no significant differences in the frequency of various B cell subpopulations between the non-responder and responder groups. Also, we showed a positive correlation between the anti-HBsAb level of responders and the frequency of IgM- MBCs in the atMBC subset. Moreover, the frequency of isotype-switched memory B cell population was significantly higher in the atMBC subset in comparison to the cMBC subset in the responder and total group.

Conclusion: There may be no immunological defects in the formation of these subsets in non-responders. It would be probable that in healthy vaccinated individuals, anti-HBsAb production may positively correlate with the level of class switching in B lymphocytes.

Keywords: Hepatitis B vaccine, Non-responders, Responders, Anti-HBsAb, Memory B cells.

Lower frequency of T stem cell memory (TSCM) cells in hepatitis B vaccine non-responders

Mahsa Eshkevar Vakili[1], Mehrnoush Doroudchi[1], Dieter Kabelitz[2], Kourosh Kalantar[3]

1. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Institute of Immunology, Christian-Albrechts University of Kiel and University Hospital Schleswig, Holstein Campus Kiel, 24105 Kiel, Germany
3. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Despite the availability of an effective vaccine and antiviral treatments, hepatitis B is still a global public health problem. Hepatitis B vaccination can prevent the disease. Vaccination induces long-lasting protective immune memory, and the identification of memory cell subsets can indicate the effectiveness of vaccines. Here, we compared the frequency of CD4+ memory T cell subsets between responders and non-responders to hepatitis B vaccination. Besides, the frequency of IFN-γ+ memory T cells was compared between the studied groups.

Methods: Study participants were grouped according to their anti-HBsAb titer. For restimulation of CD4+ memory T cells, peripheral blood mononuclear cells (PBMCs) were cultured in the presence of HBsAg and PHA for 48 hours. Besides, PMA, ionomycin, and brefeldin were added during the last 5 hours of incubation to induce IFN-γ production. Flow cytometry was used for analysis.

Results: There was a statistically significant difference in the frequency of CD4+CD95+, CD4+CD95Hi, and CD4+CD95Low/Med T stem cell memory (TSCM) cells between responder and non-responder groups. However, the comparison of the frequency of memory T cells producing IFN-γ showed no differences.

Conclusion: Our results identified a possible defect of immunological CD4+ memory T cell formation in non-responders due to their lower frequency of CD4+ TSCM cells.

Keywords: Hepatitis B vaccine, CD4+ memory T cells, Memory T cells producing IFN-γ, T stem cell memory, T central memory, T effector memory

PastoCoVac-Soberana Vaccine against COVID-19: A joint co-produced vaccine by Pasteur Institute of Iran and Finlay Institute of Cuba

Farzin Roohvand[1]

1. Molecular Virology Dept, Pasteur Institute of Iran, Tehran, Iran

Background: Pasteur Institute (PI) of Iran is one of the oldest vaccine manufacturers in the Middle East, established in 1920 in Tehran (capital), 32 years after the founding of PI-Paris in 1887. Smallpox, rabies, BCG, cholera, and anti-typhoid vaccines are some of the first-generation vaccines produced in PI-Iran. The modern "Production Complex" of PI-Iran started its activities in 1988 to produce novel 2nd-generation vaccines such as the recombinant HBV vaccine. The recombinant HBV vaccine was based on the Cuban Institute of genetic engineering and Biotechnology Technology in the production of HBsAg in Yeast. During Covid19 pandemic, PastoCoVac-Soberana (Pcov), an anti-coronavirus vaccine was jointly manufactured by IPI and Cuba's Finlay Institute of Vaccines. This is the 1st and only conjugate vaccine formulating "6xRBD (receptor binding domain) + Tetanus Toxoid (TT) conjugate formulated with Al $(OH)_3$ adjuvant".

Methods: PastoCoVac-Soberana-Plus (Pcov+), an Unconjugated Booster vaccine formulating RBD-Dimer + Al $(OH)_3$ adjuvant is also produced which is useful for boosting a pre-existing immune response in COVID-19 Convalescents and individuals with primo-vaccination with Pcov or other COVID-19 vaccine formulations. The conjugation/dimerization of RBD is based on the insertion of an extra (unpaired) cysteine at the C-terminal of the RBD protein (Cys538) and its reduction to a free thiol by tris-(2-carboxyethyl) phosphine (TCEP).

Results: Both the RBD-Dimer and 6xRBD-TT conjugate indicated proper folding and purification after expression in CHO and recognition (inhibition) by convalescent sera. In pre-clinical mice studies Pcov immunized animals indicated the highest humeral and neutralizing antibody (Nab) responses compared to other vaccine formulations. Phase I and II human studies indicated the safety and high seroconversion in immunized individuals while phase III studies indicated safety and efficacies around 70% for homologous immunization by two (25µg) doses of Pcov and 92% for heterologous immunization with two (25µg) doses of Pcov and one (50µg) booster dose by Pcov+.

Conclusion: Early studies indicated NAbs were lasting for 7-8 months after the last immunization. Results of 3rd clinical trial conducted in different cities of Iran indicated its safety and efficacy.

Keywords: COVID-19, Vaccine, PastoCoVac, Soberana

Selection of combination adjuvants for enhanced potency of a recombinant vaccine against Plasmodium falciparum: Toward precision adjuvants

Sakineh Pirahmadi[1], Hedyeh Vand Rajabpour[1], Mostafa Zargar[1], Jafar J. sani[1], Hemn Yousefi[1], Shima Afzali[1], Zeinab Pourhashem[1], Navid Dinparast Djadid[1], Sedigheh Zakeri[1], Akram Abouie Mehrizi[1]

1. Malaria and Vector Research Group (MVRG), Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran

Background: Recently, there has been considerable interest in designing combination adjuvants for malaria vaccines. Combining different TLR ligands with QS-21 has shown more promise than using a single adjuvant in recent studies. However, it is unclear whether combining TLR agonists using the same intracellular signaling pathways would have synergistic or antagonistic effects. Thus, specific combinations of TLR ligands should be evaluated for their synergistic immune stimulation in pre-clinical studies. To enhance the immunogenicity of Plasmodium falciparum cell traversal protein for ookinetes and sporozoites (PfCelTOS), we evaluated three different adjuvants; MPL (TLR4 agonist), Poly I: C (TLR3 agonist) and QS-21 in a combination of triple (MPL+Poly I: C+QS-21; MPQ) or dual (Poly I: C+QS-21; PQ) adjuvants. We assessed how dual and triple adjuvants enhance immune stimulation of PfCelTOS and whether synergistic immune activation can be achieved.

Methods: BALB/c mice were immunized with recombinant PfCelTOS admixed with dual and triple adjuvant combinations. Humoral and cellular immune responses in immunized mouse groups are evaluated and compared.

Results: Both combinations significantly increase anti-PfCelTOS antibody levels, IFN-γ and TNF-α cytokines, and functional inhibitory potency in oocyst formation in comparison to PfCelTOS alone ($p<0.05$ by one-way ANOVA). Interestingly, comparable antibody and cytokines levels and also functional inhibitory activity were found in the group that received antigen in either dual or triple adjuvants ($p>0.05$ by one-way ANOVA).

Conclusion: Our results revealed that there was no synergistic effect between TLR4 and TLR3 agonists in our vaccine formulation. This observation could be the result of the non-additive effect of combining MPL (acts through MyD88 and TRIF-mediated pathways) with Poly I: C (works through TRIF). Based on the data presented here, we would suggest that the combination of TLR3 ligand with QS-21 could be an effective adjuvant for the vaccine against PfCelTOS. This data could have a major impact on future vaccine designs that target these adjuvants.

Keywords: Combination adjuvant, Synergistic immune stimulation, Malaria vaccine, TLR agonist

Seroprevalence and geographical distribution of Bordetella pertussis antibodies in the healthy population: A systematic review and meta-analysis

Mina Alimohammadi[1], Alireza Mafi[2], Fatemeh Faramarzi[3]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Isfahan University of Medical Sciences, Isfahan, Iran.
3. Mazandaran University of Medical Sciences, Sari, Iran.

Background: Bordetella pertussis (BP) seroprevalence analysis can improve the implementation of new strategic health management such as the expanded vaccination program. Therefore, we provided a meta-analysis of the seroprevalence rate of BP infection among the Iranian population according to age, gender, and pre-and post-booster vaccination.

Methods: Systematic literature was performed in several databases to identify eligible studies evaluating the seroprevalence of specific antibodies for BP in healthy individuals. The heterogeneity test was calculated using the I2 statistic and the random-effect model. Then, pooled data were expressed as the effect size (ES) with 95% CIs.

Results: The overall IgG seroprevalence rate of BP infection in the general population was 50% (ES: 0.50, 95% CI: [0.43 to 0.57]; I2= 53.4%, p <0.0001). Furthermore, the relative rate of seropositivity was high in adults (ES: 0.45, 95% CI: [0.37 to 0.53]; I2= 28.8%, p = 0.141), children (ES: 0.54, 95% CI: [0.42 to 0.66]; I2= 63.7%, p = 0.001), pre-booster (ES: 0.34, 95% CI: [0.19 to 0.50]; I2= 44.1%, p = 0.167) and post-booster individuals (ES: 0.77, 95% CI: [0.59 to 0.94]; I2= 0.0%, p = 0.713).

Conclusion: Our findings promoted a cost-benefit immunization schedule, recommending adequate gathering information for clinical intervention targeted against BP.

Keywords: Bordetella Pertussis (BP), Seroprevalence, Vaccination, Immunity, meta-analysis

The Relationship between Vitamin D Status and Titer of anti-Hepatitis B surface antibody post hepatitis B Vaccine in the healthy adult population in Mane and Samalghan City, Iran

Hasan Namdar Ahmadabad[1], Mostafa Roshanravan[2], Zakiyeh Amini[2]

1. Department of Pathobiology and Laboratory Sciences, North Khorasan University of Medical Sciences, Bojnurd, Iran
2. Department of Nursing, Faculty of Nursing, North Khorasan University of Medical Sciences, Bojnurd, Iran

Background: Several studies have elucidated vitamin D as an important immunomodulatory factor regulating the host immune responses to different viral infections and vaccines. This study aimed to investigate the impacts of serum levels of 25(OH) D on immune responses to hepatitis B vaccination (HBV) in the healthy adult population in Mane and Samalghan City in North Khorasan, Iran.

Methods: This descriptive cross-sectional study was conducted on 134 individuals aged between 18-35 years old who were referred to health centers of Mane and Samalghan City in North Khorasan, Iran for HB vaccination. The serum levels of 25(OH) D and anti-hepatitis B surface antibody (anti-HBsAb) titer were measured through sandwich ELISA pre- and post-HBV, respectively, in the blood samples collected from partakers.

Results: Of 134 participants who were vaccinated with HBV, 132 (97%) were good responders (> 100 IU/L). It was found that there are significant differences in titer of anti-HBsAb between partakers; who were classified into three groups according to the serum levels of 25(OH) D including sufficient, insufficient, and deficient (1835 ± 252.55, 1129 ± 120.7, and 1363 ± 0.125 ng/ml, respectively, p=0.018). Our results also that serum levels of anti-HBsAb post-HBV in women and younger individuals were significantly higher than in men and older individuals, respectively.

Conclusion: Findings of this study suggest that participants with different serum vitamin D status produce seroprotective antibody titers post HB vaccination while those with sufficient vitamin D levels may produce higher titers against HBV.

Keywords: Hepatitis B surface antibody, Hepatitis B, Hepatitis B vaccine, 25-hydroxy vitamin D

The therapeutic effect of tau and Amyloid-beta vaccine in stimulating the immune system to defeat Alzheimer's disease; a systematic review and meta-analysis of the randomized clinical trials

Armin Ariaei[1], Setayesh Moradinia[2], Fatemeh Moradi[3], Auob Rustamzadeh[3]

1. Student Research Committee, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. School of Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Background: Alzheimer's disease (AD) is the most prevalent neurodegeneration disease without promising therapeutic prospects. Since toxic peptides named Amyloid-beta (Aβ) and tau have been observed in this disease, passive and active immunotherapies have been mentioned to inhibit the aggregation of toxic peptides by the mediation of the immune system, especially the phagocytose feature of microglia.

Methods: To conduct this systematic review, databases including the Cochrane Central Register for Controlled Trials (CENTRAL), PubMed, and EMBASE were evaluated. Articles dated from February 2012 to February 2023 were included in this study considering clinical trials with randomized allocation. The primary outcome was the measurement of IgG serum antibodies in the AU/ml unit over one month. Subsequently, a meta-analysis with restricted random effects mode and subgroup analysis was implemented to define the pooled effect and the intensity of immune response between treated and placebo groups through STATA 17.

Results: In the current study, a total number of four randomized clinical trial projects consisting of 559 AD patients were included. One of them utilized the tau vaccine for intervention, while the other used the Aβ vaccine. The pooled effect size of the IgG enhancement in the treat and placebo groups was 1.33 [95% Confidence Interval (CI): 0.98-1.69]. The subgroup analysis revealed the higher effect of the tau vaccine (AADvac1) compared to the Aβ vaccine (1.88 [95% CI: 1.14-2.61] and 1.24 [95% CI: 0.87-1.60]), unlike the non-significance result of the Chi-square test.

Conclusion: Since designing vaccines for therapeutic approaches in AD is a novel issue, the number of studies to justify the effect is insufficient. Moreover, long-term intervention is not possible due to some adverse effects reported by clinical trials, including encephalitis. Finally, the simultaneous outcome of Aβ and tau vaccine intervention is recommended to assess to gain better clinical results.

Keywords: Immunotherapy, Alzheimer's disease, Vaccine, Clinical Trial

The triple combination of adjuvants induces strong and functional immune responses against the CelTOS antigen of Plasmodium falciparum in BALB/c mice

Sakineh Pirahmadi[1], Mostafa Zargar[1], Zeinab Pourhashem[1], Shima Afzali[1], Jafar J. sani[1], Hemn Yousefi[1], Hedyeh Vand Rajabpour[1], Navid Dinparast Djadid[1], Sedigheh Zakeri[1], Akram Abouie Mehrizi[1]

1. Malaria and Vector Research Group (MVRG), Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran

Background: Despite decades of research, no effective vaccine against malaria has been developed. The use of combination adjuvants with recombinant antigens is considered an effective approach toward designing effective vaccines against malaria. To address the best adjuvant combinations to be admixed with Plasmodium falciparum cell traversal protein for ookinetes and sporozoites (PfCelTOS) antigen and in the completion of our earlier work, we tested the capability of a triple combination of adjuvants, MPL, Poly I: C and QS-21 (MPQ), to enhance the immunogenicity of PfCelTOS.

Methods: To achieve this goal, mice were immunized with PfCelTOS formulated with a single or combination of MPL, Poly I: C, and QS-21 adjuvants. After the second boost, anti-PfCelTOS antibodies (level, IgG isotype, and avidity) and extracellular cytokines profile were assessed using ELISA. Furthermore, the assessment of transmission-blocking activity of anti-PfCelTOS immune responses was evaluated in Anopheles stephensi using a Standard membrane feeding assay (SMFA).

Results: Our results indicated that administration of a triple combination of adjuvants with PfCelTOS enhanced the level and avidity of the anti-PfCelTOS antibodies and Th1 cytokine responses. The highest level of anti-PfCelTOS antibodies and IFN-ɣ were observed in the mouse group that received PfCelTOS with MPQ adjuvant mixture in comparison to mouse groups that received antigen with single adjuvant ($P<0.05$ by one-way ANOVA). In addition, PfCelTOS+triple adjuvants induced a significant reduction in oocyst development ($P < 0.05$ by a Mann-Whitney U test) in SMFA.

Conclusion: We conclude that the incorporation of MPQ combination adjuvants into the PfCelTOS-based vaccine may be an effective strategy to overcome the weak immunogenicity of this antigen. The triple adjuvant combination showed enhancement of antigen-specific functional antibody with an overall Th1 biased profile. Our results indicated combinatorial adjuvants with different mechanisms can stimulate better functional immune responses than adjuvants individually against malaria.

Keywords: Combination adjuvants, Malaria, PfCelTOS, SMFA

Veterinary Immunology

A comparison between adjuvant and delivering functions of zinc oxide and gelatine nanoparticles, using a chimeric protein of Brucella Omp19-Omp31

Morteza Abkar[1], Ali Torkian[1]

1. Shahid Ashrafi Isfahani University, Isfahan, Iran.

Background: The current situation with brucellosis is still problematic and uncertain, despite the fact that several studies have been conducted to attempt to eradicate the infection internationally. Microorganisms from the genus Brucella cause brucellosis, a bacterial zoonosis. Numerous studies have been conducted in the last few decades to design and develop Brucella vaccines for animals and humans that are safer and more protective due to some disadvantages shown by the current commercial vaccines. Previous studies have determined that Omp31 and Omp19 are good antigen candidates to elicit protection against Brucella spp. This study compared the immunogenicity of chimeric protein containing Omp31-Omp19 (OO) along with gelatin (gelatin/OO) and zinc oxide (ZnO/OO) nanoparticles (NPs) in mice.

Methods: After expression and purification, the recombinant OO was loaded onto gelatin and ZnO NPs. The mice were immunized by subcutaneous injection with ZnO/OO and gelatin/OO NPs. Analysis of the antibody (total IgG, IgG1, IgG2a, IgA, and IgM) and cytokine responses was performed by ELISA. Different immunized groups of mice were challenged with the virulent strain of B. melitensis 16M. Finally, lymphocyte proliferation was examined by MTT assay.

Results: The findings showed that the ZnO/OO NPs group's immune responses were much lower than those of the recombinant gelatin/OO NPs group. Both ZnO/OO and gelatin/OO NP immunization generated Th1-Th2 immunological responses, as shown by the antibody subclasses and cytokine profile. In comparison to the ZnO/OO NPs and negative control groups, the protective results demonstrated that the recombinant gelatin/OO NPs had much greater protective effects against the B. melitensis challenge.

Conclusion: The obtained results demonstrated that gelatin NPs were an effective antigen delivery system for brucellosis immunization.

Keywords: Brucellosis, Chimeric Protein, Zinc Oxide, Nanoparticles, Gelatin

A live recombinant probiotic vaccine expressing NetB toxoid could confer significant protective immunity against Clostridium perfringens infection in broiler chickens

Mohammad Ali Shamshirgaran[1], Mehdi Golchin[1]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Background: Necrotic enteritis (NE) is a devastating disease of birds caused by Clostridium perfringens (C. perfringens) type G. The NetB toxin from C. perfringens is the responsible cause of NE. After European legislation about the withdrawal of antimicrobial growth promoters from chicken's diet, several alternative approaches such as vaccination were suggested to prevent NE. To date, no commercial vaccine is available against NE. In the present study, we immunized the broiler chickens with an oral recombinant Lactobacillus casei (L. casei) expressing modified NetB toxin from C. perfringens, as a safe probiotic vaccine vector, to assess the efficacy of the vaccine against NE challenge.

Methods: The netb gene from C. perfringens (accession No. in GenBank: KY559052.1) was cloned into pT1NX expression vector after some modification, and the resultant plasmid was electroporated into the competent Lactobacillus cells. The recombinant L. casei vaccine strain expressed and displayed the NetB antigen on the cell surface, which was confirmed by western blotting and immunofluorescent assay, respectively. One-day-old broiler chickens were immunized orally with the vaccine strain on days 3, 13, and 21 for three consecutive days, and then challenged with the virulent C. perfringens on day 30 for four consecutive days. Sera from vaccinated birds were collected after each immunization, and the anti-NetB antibody responses were determined using an indirect ELISA. The individual body weights of birds were measured from day 24 to 34 at 5-day intervals. The day after the challenge experiment, birds were euthanized, necropsied, and the small intestine was examined.

Results: The birds immunized with the recombinant L. casei vaccine strain expressing the NetB toxoid showed significant protection against the experimental NE-challenge ($p<0.05$), and also induced high serum anti-NetB antibody responses to NetB protein ($p<0.05$) compared with control birds. Additionally, the vaccinated birds showed higher body weight gains during the challenge experiment compared with control groups ($p<0.05$).

Conclusion: This study showed that the L. casei vaccine strain expressing NetB toxin from C. perfringens, as a probiotic microorganism, could be a promising vaccine candidate to induce protective immunity against necrotic enteritis in broiler chickens.

Keywords: C. perfringens, Necrotic enteritis, NetB toxin, Probiotic vaccine

Advancements in Newcastle poultry heat resistance vaccines; Systematic review

Mohammad Reza Eftekhari Hasan Abad[1], Abolfazl Ghaniei[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Newcastle disease is a highly contagious viral disease affecting various bird species, including poultry. The virus can cause severe respiratory and nervous system problems and can lead to significant economic losses in the poultry industry. Vaccination is one of the most effective measures to control the spread of Newcastle disease in poultry populations. Newcastle poultry heat resistance vaccines are a type of vaccine that is developed to withstand high temperatures during storage and transportation. This study aims to evaluate the advancements in Newcastle poultry heat resistance vaccines.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on Advancements in Newcastle poultry heat resistance vaccines from January 2000 to January 2023. Twenty affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Using heat-resistant Newcastle poultry vaccines provides several advantages over traditional vaccines, including improved vaccine efficacy and reduced vaccine wastage. They can also be stored and transported without refrigeration, making them more accessible and cost-effective for poultry farmers in remote areas. The development of heat-resistant Newcastle poultry vaccines can also positively impact the cost-effectiveness of poultry vaccination programs. Traditional vaccines can be expensive to produce, store, and transport, whereas heat-resistant vaccines are typically more affordable and require fewer resources to maintain their potency.

Conclusion: The development of heat-resistant Newcastle poultry vaccines represents a significant advancement in poultry vaccination. These vaccines provide improved vaccine efficacy, reduced vaccine wastage, and increased accessibility for farmers in remote areas.

Keywords: Heat-resistant, Newcastle disease, Poultry, Systematic review

Anti-parvovirus IgG purification from dog

Mehran Bakhshesh[1], Nader Azadi[2], Mohammad Hasan Motadayen[1]

1. Razi Vaccine & Serum Research Institute
2. Dog training center of anti-narcotic police (SEPCA), Karaj

Background: Canine parvovirus is an important and highly pathogenic agent in dogs that commonly causes severe illness in puppies. The virus is distributed worldwide and despite the various vaccines being available, it is not easily be prevented and controlled. Serotherapy and, particularly, immunoglobulin therapy have been attempted as a supportive approach to cure affected puppies. In order to achieve a homologous IgG for the treatment of the fatal parvovirus infection, we tried to purify anti-parvovirus IgG from immunized dogs.

Methods: Adult dogs were immunized with a potent live parvovirus vaccine, their sera were obtained, filtered and assessed to have a high titre of anti-parvovirus IgG by using a specific quantitative commercial ELISA kit. The immune sera containing a high amount of IgG against parvovirus were then selected for IgG purification. The IgG purification was conducted in a two-stage dialysis using a dialysis tube in which various concentrations of ammonium sulfate with several incubations and centrifugations were applied in multiple steps. To ensure that the IgG was completely purified from other proteins of the dogs' sera, the purified IgGs were run on the SDS-PAGE gels.

Results: The correct and specific protein band sizes indicating purified IgG were observed on the reduced and none-reduced SDS-PAGE gels. Using Bradford protein assay, the concentrations of the purified dog IgG were calculated to be ≃30 mg/mL and their specific IgG titer against parvovirus was measured over 9000 as the threshold number of 810 was announced protective titer by the quantitative ELISA kit manufacture, Demeditec.

Conclusion: This homologous anti-parvovirus IgG which was purified through a novel, simple and inexpensive technology which is under clinical experiments, is expected to be beneficial over the commercially available heterologous immunoglobulin to treat the deadly parvovirus infection in puppies.

Keywords: Parvovirus, Immunoglobulin therapy, Iran

Assessment of Passive Immunity in Neonetal calves in Dairy herd in Mashhad suburb By Measuring Serum Total Protein

Faezeh Oskoueyan[1], Gholamreza Mohammadi[1]*

1. Department of Clinical science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: The transfer of immunoglobulins through the placenta or colostrum is called passive transfer. Cattle's placenta is syndesmochorial, which separates the contents of the mother's blood from the fetus, thereby preventing the transfer of protective immunoglobulins (Ig) from the mother to the fetus. As a result, calves are born without maternal immunity (agammaglobulin). Therefore, the development of maternal immunity in newborn calves is almost completely dependent on the absorption of maternal immunoglobulins in the colostrum3 received at birth. Inadequate intake of colostrum and insufficient absorption of colostrum immunoglobulins by newborn calves in the first hours of life leads to failure of passive transfer immunity. FPT is not a disease, it is a condition that makes newborn calves susceptible to disease and infection. The failure of passive immune transfer in newborn calves can be evaluated by measuring serum or plasma immunoglobulins (IgG) or total serum protein between 24 hours and 7 days of age.

Methods: In each herd, 12 neonatal calves aged between one and seven days were subjected to jugular venipuncture to collect blood samples. The tubes used for collection did not contain anticoagulants, as the purpose was to evaluate passive immune transfer. Following serum separation through centrifugation, the total protein was measured using a refractometer.

Results: According to the results of the McGuirk and Collins study in the year 2004, in the successful passive transfer of maternal immunity, serum total protein4 (TP) is more than 5.2 g/dLin healthy calves and more than 5.5 g/dL in sick calves. A failure of passive transfer in a herd can be indicated if the total protein value of 4 out of 12 neonatal calves falls below the cutoff.

Conclusion: In this research, a herd-based test was used to monitor the passive transfer status in neonatal calves. The results showed that herds with a suitable passive transfer status of maternal immunity had fewer neonatal diseases such as septicemia, diarrhea, pneumonia, and infectious arthritis.

Keywords: Assessment passive immunity 1, Neonatal calf 2, Colostrum 3, Serum total protein 4

Effect of extracted polysaccharides from a Chlorella vulgaris biomass on expression of interleukin-2 in broiler chicken splenocytes

Sara Mirzaie[1], Mahdi Tabarsa[2], Maliheh Safavi[1]

1. Department of Agriculture, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran
2. Department of Seafood Processing, Faculty of Marine Sciences, Tarbiat Modares University, Noor, Iran

Background: Polysaccharides isolated from algae species have been shown to have various biological properties including immunomodulatory activities. In the present study the effects of water-extractable polysaccharides from a microalgae Chlorella vulgaris on the expression of interleukin-2 (IL-2) as one of the immunostimulatory cytokines in chicken splenocytes was evaluated.

Methods: Extracted polysaccharides were fractionated using a DEAE Sepharose FF column yielding two fractions (F1 and F2). Crude polysaccharide (CP) and fractions mostly consisted of carbohydrates (71.9 to 82.9%) and protein (6 to 13.8%). The spleen is the largest peripheral lymphoid organ in chickens. In the current study, splenocytes were collected from 42 days old broiler chickens. The cell suspension was prepared in phosphate buffered saline and the suspension was layered onto Ficoll (density 1.077) to separate splenocytes.

Results: The cells were resuspended in RPMI 1640 cell culture medium, assessed for viability by trypan blue dye exclusion and were distributed to 24 well plates with polysaccharides at 200 to 1000 µg mL-1 concentrations and incubated as 26 ℃ with 5% CO2 tension for 24 h. According to the results, evaluation of immunostimulatory activities of CP and fractions revealed significant effects on chicken splenocytes interleukin-2 (IL-2) expression. The highest expression was observed for F1 at 1000 and CP at 800 µg/ml concentrations. The level of IL-2 expression by F1 was comparable to a positive control (phytohaemagglutinin). The most potent immunostimulating fraction F1, was consisted of a homogeneous polysaccharide with relatively low molecular weight, low structural compactness and mixed linkages of (1→)-, (1→3)-, (1→3, 6)-galactopyranose and -glucopyranose residues.

Conclusion: As a consequence, these polysaccharides may be considered as valuable natural compounds with the potential immunoenhancing activity in broiler chickens.

Keywords: Algal polysaccharide, Interleukin-2, Chicken splenocytes

Experimental study on mRNA expression of CD4 and CD8 in blood leukocytes of parvovirus-infected dogs with (q) PCR

Amirhossein Valafar[1], Jalil Mehrzad[1], Dariush Shirani[1], Niloofar Zarifiam[1], Milad Shahbazi Asil[1]

1. Tehran University of Medical Sciences, Tehran, Iran.

Background: Canine parvovirus type 2 (CPV-2) is one of the most common causes of viral enteritis and infectious diarrhea. This virus is highly contagious and is transmitted very quickly through mouth-nose contact with contaminated feces. Although leukopenia resulting from this disease is not found in all affected dogs, it is correlated with the severity and stage of the disease in which blood sampling was performed and is considered prognostic data for the disease. The main approach of therapy is supportively symptomatic treatment, taking into account the stages of viral infectious diseases and the role of innate immunity in the mobilization of immune cells, especially T-lymphocytes, in the pathophysiology of the disease and the lack of a detailed study regarding the changes in the expression of CD4 and CD8 genes in canine parvovirus disease. We decided to investigate the comparative changes in the expression of the mentioned genes in the T-lymphocytes of CPV-infected dogs.

Methods: In this study, we tried to find the relationship between the mRNA expression of CD4+ and CD8+ genes in T-lymphocytes and dogs infected with canine parvovirus with the quantitative (q)PCR method.

Results: After completing the tests and examining the data obtained from them, a significant decrease in the mRNA expression of CD4 and a slight decrease in CD8 was observed in the leukocytes of CPV-infected dogs.

Conclusion: Examining the results obtained with the progress of the disease, the intensity of inflammation along with the decrease in the expression of the CD4 and CD8 mRNA is associated with the knowledge of the destructive mechanism of CPV in the mitotic active precursors of circulating leukocytes and lymphoid cells, and investigating this issue in the future, perhaps by preventing this process, more effective treatment methods can be found to increase the chance of recovery.

Keywords: Canine parvovirus, CD4/CD8, inflammation, Innate immunity, qPCR, T-lymphocytes

From Invertebrates to Mammals: A Cross-Species Analysis of Innate and Adaptive Immunity

Mahdiye Azimi[1], Behzad Baradaran[2]

1. Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
2. Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Background: Comparative immunology, a very important and practical field, examines the diversity and development of the immune system in different animal species. The utility of animal models lies in their ability to facilitate studies examining differences in immune systems and response mechanisms in different species, as well as to help develop tools for new immunity against disease in humans. The main focus of the present study is the analysis and synthesis of immune systems and immune responses in a wide range of aquatic organisms, including fish, invertebrates, and mammals with emphasis on innate and adaptive immunity.

Methods: To carry out our investigation, we engaged in an exhaustive examination of the prominent PubMed database, which houses an extensive collection of current articles dedicated to examining the intricate workings of immune responses in various types of organisms, with a particular emphasis on aquatic creatures such as fish and invertebrates and mammals. We summarized the main features and mechanisms of innate and adaptive immunity in each group of animals. In addition, as a model of mammalian immunity, gene expression analysis of immunity-related genes in domestic pigs and germ-free minipigs was performed.

Results: Our research found that fish had an advanced form of adaptive immunity, with cells expressing a particular antigen receptor and immune systems like humans. Invertebrates mostly rely on innate immunity, although some have unique adaptive or adaptive-like immunities. Mammals have both innate and adaptive components to their immune system.

Conclusion: This research offers an in-depth examination of the immune mechanisms and responses that are found among fishes, invertebrates, and mammals. It showcases the extensive array of immunity systems across various animal categories, along with any evolutionary modifications about their impact on human health.

Keywords: Comparative immunology, Animal models, innate immunity, Adaptive immunity

Immunity and antibiotic use in poultry: Finding a balance; Systematic review

Mohammad Reza Eftekhari Hasan Abad[1], Abolfazl Ghaniei[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Antibiotics have been widely used in the poultry industry to prevent and treat bacterial infections. However, the overuse of antibiotics in poultry farming has led to the emergence of antibiotic-resistant bacteria, which poses a serious threat to human health. This has prompted the need to find a balance between the use of antibiotics and the promotion of immunity in poultry. This article explores the relationship between immunity and antibiotic use in poultry farming and discusses strategies for balancing the two.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on immunity and antibiotic use in poultry from January 2000 to January 2023. Twelve affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Several strategies can be used to balance immunity and antibiotic use in poultry farming. These include improving management practices to reduce the need for antibiotics, promoting alternative methods for disease prevention, such as vaccination and probiotics, and exploring genetic approaches to enhance immunity in poultry. Improving management practices can involve reducing stress on birds, improving hygiene, and optimizing nutrition. Promoting alternative methods for disease prevention, such as vaccination and probiotics, can also reduce the need for antibiotics. Genetic approaches to enhance immunity in poultry are currently being explored, and research in this area shows promising results.

Conclusion: The use of antibiotics in poultry farming has led to the emergence of antibiotic-resistant bacteria, posing a threat to human health. Finding a balance between the use of antibiotics and the promotion of immunity in poultry is crucial for poultry's and humans' health and welfare.

Keywords: Immunity, Antibiotics, Poultry, Systematic review

Immunoboosting herbal treatments for poultry diseases; Systematic review

Mohammad Reza Eftekhari Hasan Abad[1], Abolfazl Ghaniei[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Herbal treatments have long been used in traditional medicine to boost the immune system and prevent human and animal diseases. In recent years, there has been growing interest in using herbal treatments for poultry immunoboosting. This article aims to review the current literature on herbal treatments for poultry immunoboosting.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on the immunoboosting effects of herbal treatments for poultry from January 2000 to January 2023. Twenty affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Herbal treatments have been found to have immunoboosting effects in poultry. For instance, in avian influenza, various herbal therapies with Echinacea purpurea and Glycyrrhiza glabra have been shown to improve the immune response by increasing the production of anti-inflammatory cytokines, reducing lung damage, and increasing survival rates. In infectious bronchitis, herbal treatments have been reported to decrease the severity of the disease and increase the levels of anti-inflammatory cytokines, which help to reduce inflammation and tissue damage. Moreover, herbal treatments like Garlic have effectively prevented and treated Newcastle disease in poultry. Allium sativum has been shown to increase interferon-gamma production, an important mediator of the immune response against viral infections. Additionally, herbal treatments like Curcumin have been shown to have a therapeutic effect on coccidiosis, a parasitic disease affecting poultry's intestinal tract.

Conclusion: Herbal treatments have been found to have immunoboosting effects in poultry, making them a promising therapy for various poultry diseases.

Keywords: Immunomodulatory, Herbal medicine, Poultry, Systematic review

Investigating the causes of vaccination failure against Newcastle Disease and Avian Influenza in Semnan broiler flocks

Khatereh Kafshdouzan[1]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran

Background: The incidence of Newcastle disease (ND) and Avian Influenza (AI) and their economic losses have been documented despite regular vaccination programs. So, it seems imperative to analyze the causes of vaccination failure and find effective ways to prevent and control these diseases.

Methods: In this assay, 11 broiler farms located in Semnan, were randomly investigated from February 2021 to October 2022 in terms of the vaccination schedule, the incidence of ND and AI and antibody titer against these two diseases.

Results: Among 11 studied herds, 6 of them were diagnosed with AI in terms of clinical symptoms and antibody titers (3 herds after 45 days and 3 others before this age). Regarding Newcastle, 6 out of 11 herds were diagnosed with the disease.

Conclusion: Due to the fact that in all farms, ND and AI bivalent killed virus vaccines were the only ones were used, this conclusion can be drawn that in those units which gotten infected after 45 days, lack of booster dose and insufficient antibody titer caused by killed vaccines have played a role. While in the other 3 units, the high severity of the wild strains seems to be the reason for vaccination failure. Considering that the vaccine strains used in the fields are genotype 1 and 2 and the field strain in Iran is genotype 7, the vaccines do not provide sufficient protection. Another reason is the wrong method of vaccination; appropriately the live attenuated booster dose of Newcastle should get inoculated as a spray but mostly, for ease of the work, this vaccine is used in drinking water method and this significantly reduces the immunogenicity of the vaccine. Also, the proper time of inoculation in such a way that it won't interfere with the maternal antibody does not considered in Newcastle vaccination.

Keywords: Vaccination failure, Newcastle Disease, Avian Influenza

Investigating xanthogranulomatosis in psittacine; Serology, clinical, macroscopic and microscopic signs

Seyedreza Abtahi[1], Fatemeh Nasiri Dashtaki[2], Seyyed Sattar Tohidifar[3], Mohammad Karimi[4]

1. Specialist assistant of poultry clinical science, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
2. Veterinary student, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
3. Assistant professor of poultry clinical sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord ,Iran
4. Specialist assistant of clinical science, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran

Background: Xanthogranulomatosis is a rare skin disease that can be caused by infection, inflammation, bleeding, immunological disease or lysosomal and hereditary disorders.

Methods: Xanthogranulomatosis is a skin disease that can be caused by infection, inflammation, bleeding, immunological disease or lysosomal disorders or even hereditary. This form of inflammation is associated with lipometabolic disorders and is mostly seen in visceral organs, which has rarely been described in avian medicine. Xanthogranulomatosis has been reported in species such as Eclectus parrot, Budgerigar, Red-crowned parakeet, Cockatiel and etc around the world. Common clinical pathological abnormalities include leukocytosis and increased concentration of bile acids and cholesterol in the 6 months before the death of the affected bird. In the clinical examination, lesions in the form of light yellow nodules, firm, irregularly spherical, well-demarcated, pedunculated and ulcerated were observed. Histopathological examination showed multiple and diffuse infiltration of foamy macrophages, lymphocytes, multinucleated giant cells and numerous cholesterol clefts in the epiderm and derm. Xanthogranulomatosis has also been reported in wildlife birds such as the horned owl and the red-tailed hawk. Since this disease is a skin lesion, there are signs of bone tissue involvement especially the long bones of the wings and legs such as the diaphysis of the femur, humerus, radius and ulna bilaterally, as well as soft tissue such as the proximal wall of the trachea. It has been reported that this lesion is removed using a surgical method and after a period of one year, no recurrence of skin xanthoma and no health problems have been reported.

Results: Xanthogranulomatosis is a skin disease with a low prevalence in birds, which has also been reported in different species of psittacine.

Conclusion: In some cases, lesions in bone tissue and soft tissue have also been reported. Lesions are surgically removed. No recurrence has been reported.

Keywords: Xanthogranulomatosis, Psittacine, Infection, Inflammation

Investigation on the expression of IL6 and IL-1βgenes in the blood leukocytes of dogs with canine parvovirus disease by quantitative (q)PCR

Niloofar Zarifian[1], Jalil Mehrzad[1], Dariush Shirani[1], Milad Shahbazi Asil[4], Amir Valafar[1]

1. *Tehran University of Medical Sciences, Tehran, Iran.*

Background: Canine parvovirus (CPV) infection is considered as the most common pathogenic agent, which causes hemorrhagic gastroenteritis in dogs with a survival rate of about 9%. There is significantly leukopenia with a transient lymphopenia during CPV enteritis. The high mortality rate in leukopenic cases with severe could be associated with their high susceptibility to secondary superinfections. IL-6 is a pleotropic cytokine produced in response to tissue damage and infections. The first IL-6-deficient mice, which were generated in 1994, shows impaired innate-and-adaptive immune responses to viruses, bacteria and parasites. IL-1βalso increases the expression of all other cytokines like IL-6. It is considered that IL6 and IL-1βwith tumor necrosis factor (TNF)-αare one of the most important cytokines in immune modulation during-and-after infections. Study on the role of these cytokines in CPV infection is rare. Dog's immune disruption in viral diseases, like CPV disease, occurs; this might be from changes in IL-6 and IL-1β

Methods: In this study, we aimed to determine the relationship between CPV and the expression of IL6 and IL-1βgenes in the leukocytes of affected dogs by conducting clinical, para-clinical and molecular tests, especially qPCR.

Results: We observed a significant decrease in the expression of IL6 and IL-1βgenes in leukocytes of CPV-infected dogs.

Conclusion: IL6 is involved in practically all aspects of the immune system ranging from neutrophil infiltration at sites of infection to the shaping of the T-cell responses. IL-1β also increases the expression of nearly all other pro-inflammatory cytokines such as IL-6. According to these results, a relationship between leukopenia in CPV infection and secondary superinfections can be imaginable by decreased expression of these cytokines. Also, the hemorrhagic diarrhea is a consequence of endotoxemia and decreased cytokines production like IL6 and IL-1β Due to pathobiological activities of IL6 and IL-1β in various diseases, targeting these cytokines would provide new approaches in CPV therapy.

Keywords: Canine parvovirus, cytokines, Inflammation, Interleukins, Immune system, Real-time qPCR

Molecular detection of Brucella spp. infection in the horse population of Kerman

Yousof Karami[1], Elham Mohammadi[2], Mehdi Golchin[2]

1. Student of Veterinary Medicine, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Background: Brucellosis is a common disease between humans and animals with great economic losses, which has a global spread. This disease is usually asymptomatic in horses but can cause fistulous withers (septic bursitis of the supraspinous bursa), poll evil (septic bursitis of the supraatlantal bursa), arthritis, synovitis, and abortion. It is also important in terms of public health. This research was conducted to determine the frequency of brucellosis by PCR method in the apparently healthy horse population of Kerman City. The results of this research can help in improving the control and eradication process of this disease.

Methods: 100 blood samples with the anticoagulant agent were randomly collected from horse farms in Kerman City (two groups based on keeping with other animals or horses separated from other animals). Then, in order to perform the PCR reaction, the DNA of the samples was extracted. Subsequently, the PCR reaction was performed using specific primers of the Brucella genus. PCR products were electrophoresed to see the results.

Results: Brucella infection was confirmed in three samples. All three positive samples were kept with other animals (sheep and cattle).

Conclusion: Although the obtained results showed a relatively low prevalence of brucellosis in the Kerman horse population. But due to the importance of this disease in public health as well as its economic losses, serious procedures should be taken to control this disease, including not keeping different species of animals together.

Keywords: Brucellosis, Horse, PCR

Monitoring of Avian Influenza in Some Waterd Birds

Abdolreza Nabinejad[1], Nooshin Askarani[2]

1. Razi Vaccine and Serum Reserch Institute
2. Isfahan research center of teachers, Soft ware group, Technology office

Background: Influenza type A viruses are of most significance to public health due to their potential to cause an influenza pandemic. Influenza type A viruses are classified into subtypes according to the combinations of different virus surface proteins hemagglutinin (HA) and neuraminidase (NA). So far there are 18 different HA subtypes and 11 different NA subtypes such as H9N2, H5N1 and H5N8 or H7N5 as reported in birds of Iran. Aquatic birds are the primary natural reservoir for most subtypes of influenza A viruses. There are a few of aquatic birds including Coots, Swan, Pelican, Sea gull, Ducks, Flamingo and Goose in bird garden and tested for AIV by serological and molecular tests.

Methods: Using sterile siring about 2 ml of blood from the wing vein was collected and transferred for AIV titration, meanwhile the coanal cleft and cloacal swabs were prepared for the molecular test. Monthly monitoring of 5-6% of sensitive birds was examined randomly; the technical method for serology were HI, ELISA and RT-PCR for molecular and confirmation of suspected titers.

Results: All of the birds showed positive titer for H9N2 but the maximum level of antibody titer was 7 for Ducks, Goose and Swans, the lowest titer was 2 and related to pelicans, Flamingos. The sera were negative for H5N1 and H5N8 and H7 meanwhile the swabs were checked by RT-PCR technique which no one was positive.

Conclusion: This monitoring monthly was well done and sanitization of all locations and nests, gates and all areas with wide antiseptic materials was carried out daily.

Keywords: AI, Watered birds, Monitoring

Novel treatments for bovine mastitis using nanomaterials; a systematic review

Soheil Sadr[1], Babak Khoramian Toosi[1], Zahra Yousefsani[1], Hassan Borji[2]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: It is well known that mastitis is a very common disease in dairy cows, and it is also responsible for a great deal of economic loss for dairy farms as a result of this disease. Bacteria, fungi, and algae are some of the organisms that are responsible for the cause of this disease. In most cases, antibiotics are the mainstay of treatment when it comes to mastitis. Nevertheless, dairy cows have developed resistance to antibiotics as a result of the long-term use of antibiotics. As a result, alternative methods are being explored to eliminate pathogenic microorganisms that cause mastitis, which is systematically reviewed in this study.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on the treatment of bacterial mastitis with Nanoformulations from January 2000 to August 2022. Fifteen affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: In spite of this, nanotechnology is a field that is growing rapidly, providing the possibility to manufacture new materials at the nanoscale level and with the potential to revolutionize the agriculture and food industries by offering novel treatment options for prevalent and costly illnesses like bovine mastitis. It has been shown in pilot studies that the combined formula of NP and antibiotics has demonstrated great success in treating mastitis. As a result of the intra-mammary infusion of nanosilver cream combined with ceftiofur, 93.33% of the cases have an effective therapeutic result.

Conclusion: In view of the increasing ineffectiveness of current treatments for resistant bacteria, nanotechnology can be used to develop innovative products that address antimicrobial resistance as a major global issue.

Keywords: Antimicrobial resistance, Cattle, Mastitis, Nanoparticles

Ostrich Serology for ND and AI on 2021-2022

Abdolreza Nabinejad[1], Nooshine Askarani[2]

1. Razi Vaccine and Serum Research Institute,
2. Isfahan Research Center of Teachers, Soft Ware Group, Technology Office

Background: Iran's ostrich breeding industry is developing and well increased in Isfahan as the first ranking in the country, It was about 25 years ago that a few African Ostrich chicks were imported to Iran and at the moment we arrive to more than 4500 breeders just in Isfahan Province with about 6700 chicks for laying or meat production in future. One of the most complicated diseases of the ostrich is respiratory problems. The clinical signs are coupled with management and nutritional difficulties but the contagious signs of the disease propose an infectious habitat of the disease, meanwhile, biosecurity decreases the prevalence.

Methods: Sampling of blood was done via jugular and wing vein in the infected ostrich aged 2 to 6 months to 3 years from 10 farms in Isfahan from March 2021 to March 2022, The samples were transported to a serology laboratory and the sera were prepared for HI test for ND and AI and also ELISA test kit of Biochek used for chicken Aadenovirus 1 and Borna Virus investigation.

Results: Regarding the results the Adenovirus 1 and Borna virus infection were negative compared to controls both in chickens and adults, but the HI test for ND and AI were positive and valid, in which the CV for ND was 187% with the Maximum of 9, Minimum were 3 and average of 6, the CV for AI (H9N2) was 202% with the Maximum titer of 8, Minimum titer of 2 and average of 5, non of samples were positive for H5s serotypes.

Conclusion: Ostriches ND vaccination in all ages, disinfecting and biosecurity would be preventive for best and insured Ostrich breeding

Keywords: Pneumonia, AI, ND, Ostrich, Chicks, Isfahan

Production of polyclonal antibody against NetB toxin of Clostridium perfringens

Mohammad Ali Shamshirgaran[1], Mehdi Golchin[2]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Background: Clostridium perfringens (C. perfringens) is an important enteropathogenic microorganism causing necrotic enteritis (NE), a devastating enteric disease in birds. Necrotic enteritis toxin β-like (NetB) from C. perfringens type G is the causative agent of NE in broiler chickens. The NetB toxin is widely used in vaccines developed against NE. In this study, we evaluated the immunogenicity of the inactive NetB toxin (toxoid) to achieve anti-NetB antibodies for further vaccine studies.

Methods: The netb gene from C. perfringens (GenBank: KY559052.1) was modified and cloned into an expression vector (pET-22b), and the plasmid was then transformed into the Escherichia coli (E. coli) cells. The NetB protein expression in the recombinant E. coli was induced using the isopropyl-β-D-1-thiogalactopyranoside (IPTG), and the expression was confirmed by the indirect ELISA and western blotting. The NetB toxoid was then isolated from the recombinant E. coli strain. A white female rabbit was immunized intradermally with the recombinant NetB toxoid. Sera were collected from the immunized rabbit before each immunization and also two weeks after the last immunization. The anti-NetB antibody responses in the rabbit sera were determined using an indirect ELISA. The specificity of the induced anti-NetB antibodies in rabbit sera was also determined using immunoblotting.

Results: The ELISA results showed significant anti-NetB polyclonal antibodies induced in the immunized rabbit ($P<0.05$). A 33-kDa protein band developed in western blotting showed the reactivity of the polyclonal antibodies with the recombinant NetB toxoid.

Conclusion: This study showed that NetB toxin of C. perfringens is an immunogenic protein that could be served as a potent antigen in further vaccine studies.

Keywords: Antibody, C. perfringens, NetB toxin, Polyclonal

Searching for the expression of toll-like receptor 2, 4 and 9 genes in the blood leukocytes of dogs with canine parvovirus disease by Real-time quantitative (q)PCR

Milad Shahbazi Asil[1], Jalil Mehrzad[1], Dariush Shirani[1], Niloofar Zarifian[1], Amir Valafar[1]

1. Tehran University of Medical Sciences, Tehran, Iran.

Background: Canine parvovirus type 2 disease (CPV-2) is one of the most common gastrointestinal diseases of small animals, which has a high mortality and infection rate. Because there is no specific treatment for CPV, the treatment is mainly supportive treatment. Considering that the molecular part of innate immunity, especially TLRs, is vital in the defence and regulation of the host's immune responses, and they play a key role in the pathophysiology of most diseases, but there has been no study on the role of these receptors in CPV-2 in dogs in Iran and the world. According to the disruption of the dog's innate immune system in viral diseases, our hypothesis is that the canine parvovirus disease can change the level of expression of TLRs in the leukocytes of sick dogs, and maybe the disruption of the expression of these genes (exacerbation and or weakening their performance) can be a factor to accelerate the development of CPV disease in dogs.

Methods: In the present study, by conducting clinical, para-clinical and molecular tests, we tried to determine the relationship between canine parvovirus disease and the expression of TLR2, TLR4 and TLR9 genes in the leukocytes of affected dogs with quantitative (q)PCR test.

Results: After conducting tests and analyzing the obtained data, we observed a significant increase in the expression of TLR2 and TLR4 genes with a slight increase in TLR9 in leukocytes of CPV-infected dogs.

Conclusion: According to these results, a relationship between the level of inflammation in CPV and secondary infections can be expected. It can also be expected that by increasing the expression of these TLRs, such a useless inflammatory reaction is imaginable. In the future, by targeting these receptors and their signaling pathways, new approaches to CPV can be expected.

Keywords: Canine parvovirus, Inflammation, Innate immunity, Real-time qPCR, TLR

Seromonitoring of cockatoos for AI and ND in 2021-2022

Abdolreza Nabinejad[1], Nooshin Askarani[2]

1. Razi Vaccine and Serome Research Institute
2. Isfahan Research Center of Teacher, Technology Office

Background: Nowadays pet birds are going to be most popular due to changing the life cycle and more incoming humans. cockatoo is one of the birds with the most popular in new civil society and locations, in the current study some different strains of parrots which were referred to the Isfahan birds clinic will be studied by serology for ND and AI, There was no suspected clinical sign and no any vaccination done, Due to a close relationship between parrots and the owners also regarding owner request, public health and epidemiological reports the study has been done.

Methods: in the current study some different strains of cockatoo which were referred to the Isfahan birds clinic will be studied by serology for ND and AI, There was no suspected clinical sign and no vaccination done, Due to a close relationship between parrots and the owners also regarding owner request, public health and epidemiological reports the study has been done. So from 2021 April to March 2022 about 150 cockatoo were studied at the birds garden, the sampling method was Blood which was prepared using a wing vein, the sera were tested for AI and ND by HI.

Results: Regarding the results the ND titer ranged from 1 to 8, with an average of 6 and CV of 138%, The titer of the sera for H5N1 was 0 but for H9N2 were ranged from 1 to 7 and the mean titer was 5.5 with C.V. of 142.

Conclusion: The sero monitoring of parrots and pet birds must be carried out for AI and ND control and surveillance.

Keywords: Parrot, AI, ND, Seromonitoring

Significant protection of broiler chickens against necrotic enteritis after oral vaccination with the recombinant probiotic-based vector vaccine

Mohammad Ali Shamshirgaran[1], Mehdi Golchin[1]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Background: Necrotic enteritis (NE) is a serious disease in chickens caused by Clostridium perfringens (C. perfringens). The αtoxin is considered one of the most important toxins of C. perfringens to cause NE. Following the European ban on antibiotics from chicken's diet, alternative approaches including vaccination were suggested to control NE. Currently, there is no available commercial vaccine against NE. In this study, we vaccinated the broiler chickens with an oral recombinant probiotic vaccine vector, a recombinant Lactobacillus casei (L. casei) expressing αtoxin from C. perfringens to assess the efficacy of the vaccine against NE challenge.

Methods: The Carboxy-terminal fragment of αtoxin (GenBank: DQ202275.1) was cloned into pT1NX expression vector, and the resultant plasmid was electroporated into the competent Lactobacillus cells. The recombinant L. casei vaccine strain displayed the αtoxin on the cell surface and the expression was confirmed by the immunofluorescent assay and western blotting. One-day-old broiler chickens were vaccinated orally with the vaccine strain on days 3, 13, and 21 for three consecutive days, and then experimentally challenged with the virulent C. perfringens on day 30 for four consecutive days. Sera collected from birds after each immunization were evaluated for the presence of anti-αtoxin antibodies using an indirect ELISA. The body weights of birds were individually measured at 5-day intervals from day 24 to 34. The day after the challenge experiment, birds were euthanized, necropsied, and the small intestine was examined.

Results: Birds vaccinated with L. casei strain expressing the αtoxin were significantly protected ($p< 0.05$) against the experimental NE, and also elicited high antitoxin antibody responses to α toxin ($p< 0.05$). Furthermore, the immunized birds showed higher body weight gains during the challenge experiment compared with control birds ($p < 0.05$).

Conclusion: This study showed that a probiotic bacterium, L. casei expressing αtoxin from C. perfringens, could be a promising vaccine candidate to protect chickens against NE.

Keywords: C. perfringens, Necrotic enteritis, Probiotic, Vaccine

Subcutaneous vaccination with Urease-omp19 loaded chitosan and gelatin nanoparticles elicit high protection against Brucella melitensis

Masoumeh Sadat Derakhsh[1], Zahra Zamanzadeh[1]

1. Department of Genetics, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani University, Isfahan, Iran

Background: Brucellosis or malt fever is one of the most important zoonotic diseases in animals and humans. The results of brucellosis include abortion, reduction of milk production and infertility in infected animals and transmission probability to human. The disease caused by Brucella spp as facultative intracellular bacteria. Due to the disadvantages of commercial vaccines, there is an urgent need to design effective vaccines against the disease. Previous studies have shown that Omp19 and Urease can result in protection against B. abortus, B. melitensis. In this work, we evaluated the vaccine potential of chitosan and gelatin nanoparticles (NPs) formulation of a chimeric protein containing Om19 and Urease (UO) against brucellosis in mice.

Methods: Expression and purification of recombinant protein, nanoparticle preparation, characterization of antigen-loaded chitosan and gelatin NPs, mice immunization, antibody assay, cytokine quantitation and protection assay were performed.

Results: Based on the cytokine profile and subclasses of the antibody, subcutaneous vaccination with both UO load chitosan (Ch/UO) and UO load gelatin (Ge/UO) NPs induced T helper type 1 (Th1)-T helper type 2 (Th2) immune response. Vaccinated groups of mice when challenged with B. melitensis 16M were found to be significantly protected in the Ge/UO NPs administered group in comparison with the Ch/UO NPs immunized mice. Ge/UO NPs elicited protection toward B. melitensis challenge equivalent to the commercial vaccine strain B. melitensis Rev.1.

Conclusion: Altogether, our results indicated that gelatin nanoparticles along with UO antigen are a promising vaccine candidate against B. melitensis.

Keywords: Urease, brucellosis, gelatin, Omp19

Surveillance for AIVs (H9 & H5s) in Isfahan Birds Garden

Abdolreza Nabinejad[1], Nooshin Askarani[2]

1. Razi Vaccine and Serum Research Institute
2. Isfahan research center of teachers, Soft ware group, Technology office

Background: Isfahan bird's garden is located at the Zayandeh rood river and with about 130 specious and a number of 3000 different birds, last year in covid 19 epidemy about than 300000 people of around the country and tourists visit the birds. Regarding to HP AIs (H5N1 and H5N8) outbreak in the poultry farms (industrial and rural) and immigration of immigrant birds to the central part of the Iran ,a passive surveillance were carried out, for increasing immunity of the for the visitors and birds.

Method:A daily shuttle program for sanitization of garden and gates with widely antiseptic material were recommended, and an active quarantine for a sure immunity, meanwhile educational techniques used for training and refreshing the staffs specially the birds nurses which are at the first line of communication to the birds ,In the laboratory examination a monthly monitoring for NDV and AIV titer well done by HI test ,so 2 ml of wing vein blood in 5-6% of sensitive birds were collected and some swabs of pharynx and stools were prepared in the transporting media for RT-PCR(in suspected birds) molecular detection.

Result: The mean ND titer were 6 with CV of 68% and the mean titer of H9N2 were 5 with a CV equal to 87%, but the highest ND titer (9) were related to poultry and pigeons, the lowest ND titer(3) were related to water fowls such as Ducks, Pelicans , Flamingos ,Gooses . The minimal H9N2 titer were 4 and the maximal value were 8 but no one were positive for In H5N1 and H5N8 .The molecular RT-PCR of samples for M proteins of H5 and H7 were negative.

Conclusion: Regarding to the result a monthly vaccination for ND and AI and continues first level of biosecurity were recommended. Also using the training and intelligent board inside and outside the garden with some regular education course were the assessment of this survillence.

Keywords: AIVs, Birds, Isfahan, Biosecurity, Vaccination

The application of nano-vaccines in veterinary medicine: a systematic review

Ashkan Hajjafari[1], Soheil Sadr[2], Fateme Jalili Shahri[2], Pouria Ahmadi Simab[3], Hassan Borji[4]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University, Science and Research Branch, Tehran, Iran
2. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Pathobiology, Faculty of Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
4. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: These days, effective and safe vaccination illustrates cost-effective and strong economic development and international health techniques. In veterinary medicine, vaccination is necessary to enhance public health and defeat zoonoses. Nevertheless, there is a need for new vaccination systems to manage persistent infective, rapidly growing, and emerging/re-emerging pathogens for which no helpful vaccine has been designed. Nanoscience in veterinary medicine particularly pays attention to three principal factors: diagnosis, vaccination, and treatment. This study will discuss Nano-Biotech programs related to vaccination science.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on Nano-Vaccinology in Veterinary Medicine Science from January 2000 to January 2023. Twenty-five affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Nano vaccines are successful because of the enhanced lowest Immunotoxicity, antigen stability, better immunogenicity, the flexibility of the physical characteristics of nanomaterials, and sustained release. Nano vaccines are more efficient than ordinary vaccines due to ease of control and plasticity in physio-chemical properties. Based on Nano-Vaccination, both humoral immunity and cellular responses can be invoked. By engineered Nano-Vaccines, highly targeted immunological pathways may be achieved, which is required for long-lasting and solid immunity. Some of Nano-Vaccines' positive features are controlled drug release, increased drug stability, and improved targeting capacity. Antigen-carrying Nano-Particles can influence the immune system and improve the T-cell cytotoxic response against antigens linked to Nano-Particle.

Conclusion: This study article uses Nano-Vaccinology to avoid infectious diseases such as viral, bacterial, parasitic, and noninfectious ones, including auto-immunity and cancer. In addition, as a future research field, successful application challenges from bench to clinical usage have been highlighted.

Keywords: Nano-Vaccine, Nanotechnology, Veterinary, Zoonosis Control

The benefits of probiotics and prebiotics for poultry immune health; Systematic review

Mohammad Reza Eftekhari Hasan Abad[1], Abolfazl Ghaniei[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Probiotics are live microorganisms that can confer health benefits to the host when administered in adequate amounts. Prebiotics, however, are non-digestible food ingredients that selectively stimulate the growth and activity of beneficial bacteria in the gut. Probiotics and prebiotics have been gaining popularity in the poultry industry for their potential to support gut health and improve immune function. This article will explore the benefits of probiotics and prebiotics for poultry immune health and how they can help improve overall poultry health and productivity.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on the benefits of probiotics and prebiotics for poultry immune health from January 2000 to January 2023. Twenty affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: The gut microbiota plays a crucial role in maintaining a healthy immune system in poultry. Probiotics and prebiotics promote the growth of beneficial bacteria in the gut and reduce the population of harmful bacteria. This can help maintain a balanced gut microbiota, essential for proper immune system function. Probiotics and prebiotics can help improve immune function by promoting the production of immune cells, regulating inflammation, and increasing antibody production. Probiotics and prebiotics have been shown to reduce the incidence of necrotic enteritis, coccidiosis, and Salmonella in poultry. Probiotics and prebiotics have improved poultry growth, feed efficiency, and egg production.

Conclusion: In conclusion, probiotics and prebiotics can support poultry immune health and improve overall poultry health and productivity. By promoting healthy gut microbiota, these beneficial microorganisms can help reduce disease incidence and improve immune system function.

Keywords: Probiotics, Prebiotics, Poultry, Systematic review

The potential of combination nanotechnology and immunology in the treatment of poultry diseases: Systematic review

Mohammad Reza Eftekhari Hasan Abad[1], Abolfazl Ghaniei[2]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Poultry diseases have been a major concern for the poultry industry, leading to significant economic losses. The combination of nanotechnology and immunology has shown potential in treating poultry diseases. This review aims to summarize the potential of nanotechnology and immunology in treating poultry diseases.

Methods: Nine databases (PubMed, Scopus, Google Scholar, Cochrane Library, Magiran, SID, Medline, Embase, and Lilacs) were searched for published articles on nanotechnology and immunology in the treatment of poultry diseases from January 2000 to January 2023. Twenty affiliated articles with complete abstracts were included in this study. All data were extracted from interconnected papers and analyzed with R version 4.2.1 artificial intelligence software.

Results: Nanoparticles have been used to treat various poultry diseases, including avian influenza and Newcastle disease. Nanoparticles can be engineered to specifically target infected cells and deliver drugs, such as antiviral agents and vaccines, to enhance their efficacy. For instance, liposomal nanoparticles have been used to deliver interferon-alpha to infected cells, significantly reducing the severity of avian influenza. Similarly, polymeric nanoparticles have been used to deliver Newcastle disease vaccines, significantly increasing antibody production and protection against the disease. The combination of nanotechnology and immunology has also shown potential in treating poultry diseases. Immunomodulatory nanoparticles, such as mesoporous silica and chitosan nanoparticles, have enhanced the immune response and reduced the severity of various poultry diseases.

Conclusion: Combining nanotechnology and immunology has shown significant potential in treating poultry diseases. Using nanoparticles to deliver drugs and enhance the immune response can provide an effective and sustainable alternative to antibiotics for treating these diseases.

Keywords: Immunomodulatory, Nanotechnology, Poultry, Systematic review

Using Garlic (Allium sativum) powder for improving immunity and t infections treating in broilers

Abdolreza Nabinejad[1], Alireza Nabinejad[2], Nooshine Askarani[3]

1. Razi Vaccine and Serum Reserch Institute
2. RUDN University of Dentistry
3. Isfahan Research Centre of Teachers, Software Group, Technology office

Background: Poultry industry is the most financial system in Iran after the oil, its importance for meat and protein production for food insurance and jobs creation is increasing currently, unfortunately some of infectious disease included viral and bacterial besides the environmental changes and bad quality of farms and diets, affected the poultry farms special in broilers.

Methods: Using of antibiotics shows some side effects such as bacterial resistance and increasing the costs of production, meanwhile some times it is necessary to use more than one antibiotics as mass treatment simultaneously, some bacteria like as E.coli, Motile salmonella, Pseuodbacteria and Kelebciella isolated from broilers may be resistant to Tetracyclines, Doxycycline, Erthromycin , Florfenicol, Lincomycin ,and Colistin.

Results: Regarding to antibacterial activity of Garlic (Allium sativum) against G+ and G- bacteria, usage of garlic powder proposed for some farms infected by superior bacteria next to the antibiotic,so garlic powder were added 1% in the diets for 2 to 3 days around infection and just garlic during vaccination time.

Conclusion: Our observations in the farms used the garlic powders with or without other antibiotics, bacterial infection were controlled and the efficiency ratio were better than the others, in the slaughter houses the carcasses were better and the omitted ones were less than the others Meanwhile the vaccination result were increased.

Keywords: Garlic, Broiler, Infection, Immune, Stimulation

Vaccination against Newcastle disease using an apathogenic heat-resistant V4 vaccine

Mozhgan Moghani[1], Seyed Ali Ghafouri[1], Abolfazl Ghaniei[1], Soheil Sadr[1]

1. Department of Clinical Science, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Background: Newcastle disease is one of the most important and deadly common poultry diseases in Iran and the world. Until now, the implementation of various vaccination programs has not been able to prevent the occurrence of disease and deaths in some cases. NDV4HR vaccine is a non-pathogenic, heat-resistant live vaccine that, despite being non-pathogenic, can induce immunity in vaccinated poultry. This study investigates the effectiveness of the NDV4HR vaccine in broiler poultry.

Methods: A total of 60 one-day-old broiler chickens of a local hybrid available in Iran (Arian) were obtained and were divided into three groups of 20. Group A had the usual vaccination program in broiler flocks, group B did not receive any vaccine, and group C received the NDV4HR vaccine weekly from the first day to the 35th day of breeding. Blood samples were collected weekly from birds of each group and subjected to HI. Various indicators, such as weighing and feed conversion ratio, and necropsy lesion score, were recorded weekly.

Results: Regarding blood titer, Groups A and C had higher titers than Group B. There was no significant difference in HI titers between Group A and Group C during the experiment. Regarding weight gain, there was no significant difference among the groups.

Conclusion: In this study, we concluded that the broiler flocks that used the heat-resistant NDV4HR vaccine acquired good immunity compared to the vaccinated poultry with the usual vaccination program.

Keywords: Broiler, Heat-resistance, Newcastle disease, Vaccination