Original Article

Iranian Journal of Immunology

June 2025; 22(2):142-154.

Altered Serum IL-35 Levels and IL-37 Gene Expression in Patients with Parkinson's disease: Focus on Emerging Cytokines

Fariba Akbari Gavabari[1], Mohsen Rastegari-Pouyani[1,2], Saeid Afshar[3,4], Mehrdokht Mazdeh[5], Elaheh Talebi-ghane[6,7], Mohammad Mahdi Eftekharian[1,8*]

[1]Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; [2]Research Center for Molecular Medicine, Institute of Cancer, Avicenna Health Research Institute, Hamadan University of Medical Sciences, Hamadan, Iran; [3]Cancer Research Center, Institute of Cancer, Avicenna Health Research Institute, Hamadan University of Medical Sciences, Hamadan, Iran; [4]Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Hamadan University of Medical Sciences, Hamadan, Iran; [5]Department of Neurology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran; [6]Modeling of Noncommunicable Diseases Research Center, Institute of Health Sciences and Technologies, Avicenna Health Research Institute, Hamadan University of Medical Sciences, Hamadan, Iran; [7]Clinical Research Development Unit of Fatemieh Hospital, Hamadan University of Medical Sciences, Hamadan, Iran; [8]Neurophysiology Research Center, Institute of Neuroscience and Mental Health, Avicenna Health Research Institute, Hamadan University of Medical Sciences, Hamadan, Iran

ABSTRACT

Background: Parkinson's disease (PD) is increasingly recognized as a condition driven by both central and peripheral inflammatory responses, largely mediated by cytokine activity.

Objective: To assess IL-35 (P35 and Ebi3 subunits) and IL-37 gene expression, along with the serum levels of IL-35 protein in patients with PD compared to healthy controls.

Methods: Cytokine gene expression was measured using the qRT-PCR technique, while IL-35 serum levels were measured using the ELISA method. The data obtained were analyzed using a Bayesian regression model in the R software.

Results: The results revealed that the expression of P35 gene, of the two subunits of IL-35, did not differ significantly between the two groups. However, Ebi3 and IL-37 transcript levels were significantly lower in patients compared to healthy individuals (p<0.001). In contrast, IL-35 serum level in patients showed a significant increase compared to the control group (p=0.016). Notably, IL-37 expression showed a negative correlation with age (p=0.004). We also observed positive and significant correlations between the gene expression of P35 and Ebi3 (p=0.02, r=0.4), P35 and IL-37 (p=0.008, r=0.45), and Ebi3 and IL-37 (p=0.016, r=0.41).

Conclusion: In conclusion, our study revealed a higher serum protein level of IL-35 in PD patients compared to the healthy control group. Meanwhile, gene expression levels of IL-37 and Ebi-3 were significantly reduced. These alterations in the expression of these cytokines are suggested to be partly responsible for the immune system dysregulation in this disease.

Keywords: Interleukin-35, Interleukin-37, Inflammation, Parkinson's disease

*Corresponding author:
Mohammad Mahdi Eftekharian, Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Fahmideh Blvd., Hamadan, Iran
Email: eftekharian@umsha.ac.ir

Cite this article as:
Akbari Gavabari F, Rastegari-Pouyani M, Afshar S, Mazdeh M, Talebi-ghane E, Eftekharian MM. Altered Serum IL-35 Levels and IL-37 Gene Expression in Patients with Parkinson's disease: Focus on Emerging Cytokines. *Iran J Immunol.* 2025; 22(2):142-154, doi: 10.22034/iji.2025.105508.2955.

Received: 2025-01-11
Revised: 2025-04-13
Accepted: 2025-04-14

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD). PD incidence increases from the sixth to the ninth decade of life (1). This disease is caused by the accumulation of Lewy bodies and destruction of dopaminergic neurons in the substantia nigra of the midbrain, leading to significant movement disorders (2).

While aging remains a primary risk factor for PD development, both genetic predisposition and environmental exposures also contribute significantly to disease pathogenesis (3). Historically classified as a movement disorder due to of its characteristic motor symptoms such as tremors and bradykinesia, PD is now recognized as a multi-system disease involving immune dysregulation and neuroinflammation (4). Growing evidence demonstrates that both central and peripheral inflammatory processes play crucial roles in the pathogenesis of PD (5). Central inflammation PD arises from dysregulated microglial activity of, astrogliosis and CNS-infiltrating activated T cells, driving excessive production of pro-inflammatory cytokines. Concurrently,peripheral inflammation is evidenced by the elevated serum levels of pro-inflammatory cytokines such as IL-6, TNF-α and IL-2 in PD patients (5). Notably, clinical studies demonstrate a direct correlation between increased inflammatory markers in the blood and cerebrospinal fluid (CSF) with progressive worsening of motor and non-motor symptoms PD patients (6).

Interleukin 35 (IL-35) is an emerging cytokine that plays an anti-inflammatory role and has not yet been investigated in the context of PD. This cytokine belongs to the IL-12 family and consists of two subunits, P35 and Ebi3 (Epstein-Barr Virus-induced gene 3), exerting its biological effects through STAT1 and STAT4 signaling pathways (7). IL-35 subunits are shared with other IL-12 family cytokines, such as the P35 subunit combining with P40 to form IL-12, and Ebi3 combining with P28 to form IL-27 (8). IL-35 is expressed in a variety of tissues including blood, bone marrow, thymus, and liver (9). In addition to stimulating the production of IL-10, this cytokine can inhibit T cell polarization towards T helper 2 (Th2) cells. It also inhibits the secretion of Th2-related cytokines, including IL-4, IL-5 and IL-13 (10). Of note, IL-35 reduces Th17 cell activity by modulating the IL-23/IL-27 ratio, as well (11). Studies have shown that IL-35 can protect against the development of some autoimmune inflammatory diseases such as encephalomyelitis and multiple sclerosis by expanding regulatory B (Breg) cells (12).

Another emerging immunomodulatory cytokine is IL-37, a member of the IL-1 family, plays a role in regulating and inhibiting inflammation. It exerts anti-inflammatory effects by suppressing NF-κB and MAPK signaling pathway while simultaneously activating the Mer-PTEN-DOK axis (13). IL-37 has five isoforms, including IL-37a, IL-37b, IL-37c, IL-37d and IL-37e that are synthesized as inactive precursors and require caspase-1-mediated cleavage for biological activation (14). This cytokine is produced at low levels in human peripheral blood mononuclear cells (PBMCs) and can be induced by inflammatory stimuli and cytokines such as IL-1, IL-18, TNF-α, interferons (IFNs), and transforming growth factor-beta (TGF-β) (15). It has been observed that IL-37 expression is increased in PBMCs of patients with multiple sclerosis particularly during disease exacerbation (16). This cytokine suppresses innate immune responses through the inhibition of the NOD family of receptors like the NLRP3 component of the inflammasome complex (17). It can also induce tolerance in dendritic cells, thus inhibiting acquired immunity (18).

In this current study, we aimed to evaluate the gene expression of IL-35 (P35 and Ebi3 subunits) and IL-37, as well as serum levels of IL-35 protein in PD patients compared to healthy controls

MATERIALS AND METHODS

Study Groups

A total of 68 individuals participated in this study, including 34 patients with PD and 34 healthy individuals who were matched in terms of age and sex. The definitive diagnosis of the disease and the classification of patients into three categories -mild, moderate, and

severe - based on the criteria of "Adams and Victor's Principles of Neurology", were performed by a neurologist. Inclusion criteria for patients were being between the ages of 50 and 80, suffering from PD and receiving L-dopa + Carbidopa as an anti-PD treatment. Exclusion criteria included suffering from any metabolic, autoimmune, immunological or neurological disorder other than PD. In the case of the healthy group, meeting the selection criteria included not having any neurological, immunological, or autoimmune diseases and being between the ages of 50 and 80 years old. The study followed the ethical standards set by the Institutional and/or National Research Committee (INRC) and adhering to the principles of the 1964 Helsinki Declaration and its subsequent amendments. Approval was granted by the Ethics Committee of Hamadan University of Medical Sciences with the ethics code *IR.UMSHA.REC.1402.625*. All participants completed and signed the informed consent form.

RNA Extraction and cDNA Synthesis

In the present study, whole blood samples were collected from all participants, including patients with PD and a healthy control group, and stored in tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). White blood cells (WBC) were then separated from the samples and used for total RNA extraction. RNA extraction was performed using RNA-X Plus extraction solution (Sinaclon Company, Tehran) according to the manufacturer's protocol. To quantitatively and qualitatively evaluate the extracted RNA, a Nanodrop device (Thermo Fisher Company, USA) and agarose gel electrophoresis were used, respectively. These evaluations were conducted to verifythe integrity and purity of the extracted RNA and optimize the conditions for the synthesis of complementary DNA (cDNA). In the next step, 2 to 6 micrograms of the extracted total RNA were used for cDNA synthesis using a cDNA synthesis kit (Parstous Company, Tehran, Iran).

Quantitative Real-time PCR (qRT-PCR)

In this study, Ampliqon™ 2X Real-Time PCR Master Mix Green without ROX (AMPLIQON, Odense, Denmark) was used to evaluate the expression of P35, Ebi3 (for IL-35) and IL-37 genes. The qRT-PCR temperature program was set as follows: one pre-incubation cycle at 95 °C for 15 minutes, then three amplification steps including 40 cycles of 95 °C for 15 seconds, 57 °C (P35), 62 °C (Ebi3), 51 °C (IL-37) and 54 °C (GAPDH) for 30 seconds and finally 72°C for 30 seconds. All reactions were performed using Light Cycler 96 (Roche, Germany), and normalization of data was done using GAPDH gene as a control gene (housekeeping gene). The gene expression level was calculated using the $Efficiency^{-\Delta\Delta CT}$ formula. In Table 1, the specifications of all primers (TAG Copenhagen, Denmark) are presented.

Enzyme-linked Immunosorbent Assay (ELISA)

Peripheral blood from the participants was collected using gel tubes and after centrifugation, serum was separated. The sera were stored at -70 °C until use. IL-35 levels were measured using the Human high-sensitive Interleukin 35 (hsIL-35) ELISA kit (Zellbio GmbH, Germany), which utilizes a biotin double antibody sandwich technology.

Table 1. Specifications of the primers used in this study.

Gene	Primer sequence	Primer length	Product size	Accession number
P35	F: TGTACCAGGTGGAGTTCAA R: CAATAGTCACTGCCCGAAT	19 19	231 bp	NM_001397992.1
Ebi3	F: TTCATAACAGAGCACATCATC R: CTCCCTGACGCTTGTAAC	21 18	160 bp	NM_005755.3
IL-37	F: TCTCTACTGTGACAAGGATAAAGG R: CGACTCCAGCATGTTCCAG	24 19	151 bp	NM_014439.4
GAPDH	F: CCATCACTGCCACCCAGAAGAC R: ATGACCTTGCCCACAGCCTTG	22 21	124 bp	NM_001357943

In summary, 40 µL of serum sample, 10 µL of biotinylated anti-hsIL-35, and 50 µL of streptavidin-HRP were added to plate wells pre-coated with anti-hsIL-35 monoclonal antibodies. After 60 minutes of incubation at 37 °C, the wells were washed 5 times with diluted washing solution. Next, 100 µL of chromogen solution was added to each well, followed by a 10-minute incubation at 37 °C for color development. Finally, 50 µL of stop solution was added to stop the reaction, and OD values were immediately read at 450 nm. IL-35 concentrations of samples were calculated (in pg/mL) based on the standard curve drawn according to standards' concentrations and the corresponding OD values.

Statistical Analysis

In this study, IL-35 serum levels were found to have a normal data distribution. However, due to the non-normal data distribution of f P35, Ebi3, and IL-37 genes expression, the logarithm (Ln) of the data was taken (19). A Bayesian regression model was employed to compare gene expression levels and IL-35 serum concentration between the two groups, in gender subgroups, and to identify potential correlations between different variables. The Bayesian regression approach allows for the integration of prior knowledge into statistical modeling by incorporating prior distributions, which then generate full posterior distributions for the parameters of interest. This method is particularly advantageous when dealing with small sample sizes, as it leverages strength from prior distribution to produce more accurate estimates. In this study, Monte Carlo chains with 6000 iterations and 1000 warm-up steps were used to evaluate Bayesian regression parameters, enabling the computation of p-values and 95% credible intervals (95% CrI). Unlike traditional frequentist methods, Bayesian inference offers probabilistic interpretations of parameter estimates, enhancing statistical robustness and predictive reliability. ROC curves were plotted to predict the disease state and determine the index for differentiating between the case and control groups.

RESULTS

Demographic Characteristics of Participants

In this study, a total of 34 patients and 34 healthy individuals, matched for age and sex, were included. The mean ages for the patient and control groups were 67.21±6.74 and 66.91±8.5 years, respectively. The demographic information of the participants were shown in Table 2. Additionally, the clinical symptoms of PD patients are outlined in Table 3.

Gene Expression Analysis and ELISA

In this study, the levels of IL-37 and Ebi3 gene expression were found to be significantly higher in the control group compared to the patients group. The results of Bayesian regression analysis (Table 4) revealed that the control group had 1.16 units higher Ebi3 expression and 1.06 units higher IL-37 levels compared to patients (p<0.001 for both).

Table 2. Demographic data of the studied groups presented as number or mean±SD

Variables	Patients				lHealthy subjects	*P*-value
Female/male (no. (%))	6 (17.6%)/28 (82.4%)				6 (17.6%)/ 28 (82.4%)	0.999[(a)]
Age range (year)	50-80				49-80	0.95[(b)]
	Total	Mild	Moderate	Severe		
Age (mean±SD, year)	67.2±6.74	66.67±5.0	69.25±7.6	66.50±7.7	66.91±8.5	0.875(Total patient vs. HC) 0.631(Patients subgroups)
Disease duration range (year)	1-21	4.83±3.6	8.44±6.7	9.36±5.9	-	0.108
Age at Diagnosis range (year)	44-73	61.83±5.1	60.81±6.9	57.14±7.3	-	0.176

[a]Chi-square Test; [b]Independent t-test

Table 3. Clinical Characteristics of the Patient Group presented as number and percentage

	Patients (n=34)	
	Number	Percentage
Tremor	28	82%
Bradykinesia	21	61%
Muscle rigidity	18	53%
Foot dragging	21	61%
Retropulsion	13	38%
Trunk flexion	21	61%
Urinary frequency	14	41%
Gastrointestinal disorder	17	50%
Depression	16	47%
Sleep disturbances	18	53%
Hallucinations and delusion	9	26%
Paraesthesia	9	26%
Dysphagia	12	35%
Sialorrhea	5	14%
Soft speech	20	58%

Specifically, a significant increase in Ebi3 expression was exclusively detected in men (posterior Beta=1.32; $p<0.001$), whereas a significant increase in IL-37 expression was observed in both genders ($p<0.001$ for men and $p=0.047$ for women). Furthermore, age was inversely associated with IL-37 expression, with levels decreasing by 0.04 units per additional year of age ($p=0.004$). This association was particularly pronounced in men (posterior Beta=1.13; $p=0.001$) (Table 4 and Fig. 1).

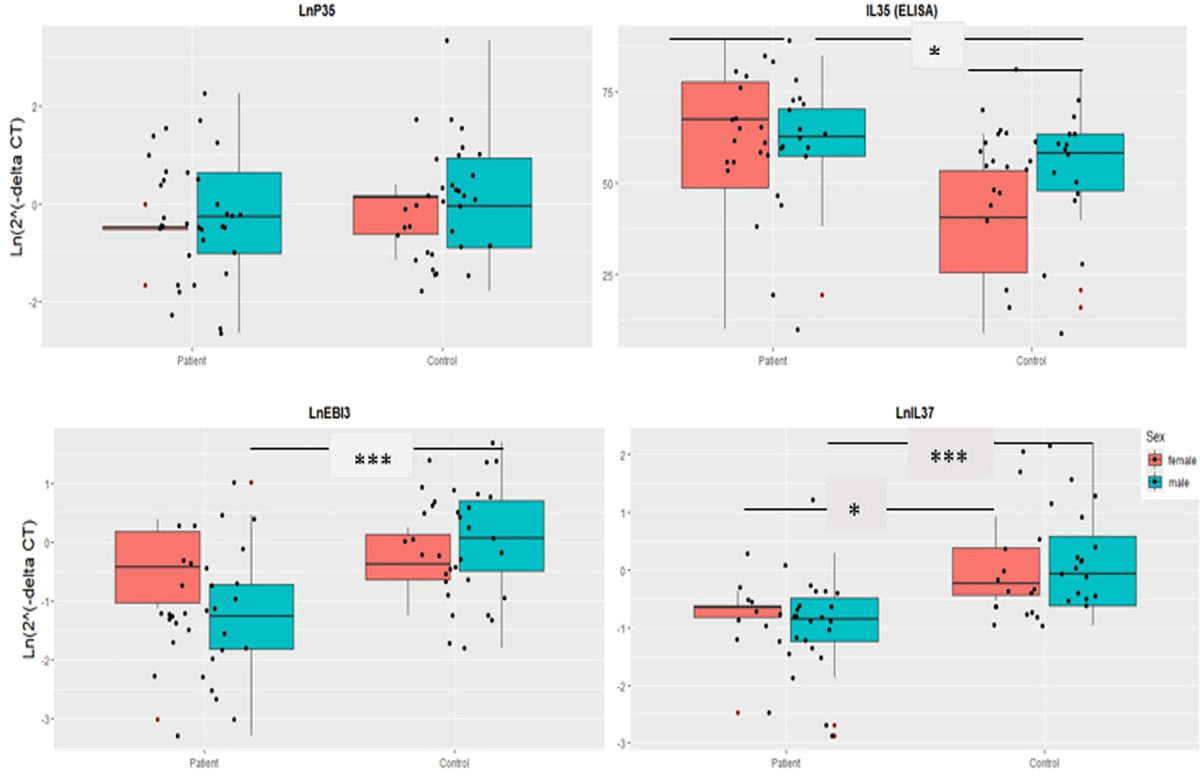

Fig. 1. Comparison of the relative expression of P35, Ebi3 and IL-37 genes, as well as serum levels of IL-35 in PD patients and healthy controls based on gender using independent t-tests. (*p=0.01-0.05, ** p=0.01-0.001, *** p<0.001). Box plots was used to display the data distribution, with the box representing the interquartile range (IQR), the line inside indicating the median, and the whiskers extending to the most extreme data points within 1.5 times the IQR.

Table 4. Relative expression of P35, Ebi3 and IL-37 genes, as well as serum levels of IL-35 in PD patients and healthy controls based on the results of the Bayesian regression model.

Variable		LnP35					IL35 (ELISA)				
		Posterior Beta	SE	95% CrI	Standardized Effect Size	*P*-value	Posterior Beta	SE	95% CrI	Standardized Effect Size	*P*-value
Total	Group (Control/Case)	0.27	0.29	[-0.29, 0.83]	0.28 [-0.28, 0.84]	0.328	-9.78	4.02	[-17.61, -1.87]	-8.45 [-15.76, -1.17]	0.016
	Gender (Male/female)	0.36	0.37	[-0.35, 1.1]	0.36 [-0.38, 1.09]	0.315	9.83	5.31	[-0.53, 20.14]	8.53 [-1.34, 18.06]	0.063
	Age	-0.03	0.02	[-0.07, 0.01]	-0.24 [-0.51, 0.04]	0.101	0.03	0.27	[-0.49, 0.56]	0.21 [-3.46, 3.96]	0.903
Male	Group (Control/Case)	0.28	0.33	[-0.38, 0.94]	0.28 [-0.41, 0.95]	0.393	-7.69	3.72	[-15.05, -0.31]	-7.67 [-15.05, -0.40]	0.039
	Age	-0.03	0.02	[-0.08, 0.01]	-0.24 [-0.57, 0.09]	0.156	0.30	0.25	[-0.18, 0.78]	0.30 [-0.20, 0.79]	0.218
Female	Group (Control/Case)	0.28	0.39	[-0.48, 1.05]	0.27 [-0.52, 1.09]	0.455	-26.53	15.47	[-56.77, 5.84]	-7.86 [-23.80, 8.93]	0.087
	Age	-0.03	0.02	[-0.07, 0.02]	-0.22 [-0.63, 0.20]	0.249	-1.36	0.99	[-3.32, 0.64]	-3.31 [-11.45, 5.32]	0.151
		LnEBI3					LnIL37				
Total	Group (Control/Case)	1.16	0.24	[0.67, 1.64]	1.16 [0.69, 1.63]	<0.001	1.06	0.20	[0.66, 1.45]	1.05 [0.65, 1.45]	<0.001
	Gender (Male/Female)	-0.14	0.32	[-0.77, 0.49]	-0. 14 [-0.77, 0.48]	0.652	0.17	0.26	[-0.35, 0.69]	0.17 [-0.36, 0.71]	0.509
	Age	-0.01	0.02	[-0.04, 0.02]	-0.09 [-0.33, 0.14]	0.455	-0.04	0.01	[-0.06, -0.01]	-0.29 [-0.50, -0.09]	0.004
Male	Group (Control/Case)	1.32	0.25	[0.82, 1.82]	1.32 [0.81, 1.81]	<0.001	1.13	0.23	[0.69, 1.57]	1.13 [0.68, 1.57]	<0.001
	Age	-0.03	0.02	[-0.06, 0.01]	-0.19 [-0.44, 0.06]	0.126	-0.05	0.02	[-0.08, -0.02]	-0.05 [-0.08, -0.02]	0.001
Female	Group (Control/Case)	0.71	0.75	[-0.79, 2.19]	0.70 -0.85, 2.20]	0.313	0.99	0.47	[0.03, 1.91]	1 [0.04, 1.96]	0.047
	Age	0.03	0.05	[-0.06, 0.13]	0.28 [-0.53, 1.05]	0.427	0.01	0.03	[-0.05, 0.07]	0.1 [-0.4, 0.62]	0.671

SE: Standard error; CrI: Credible intervals; Ln: log10 ($2^{-\Delta\Delta Ct}$)

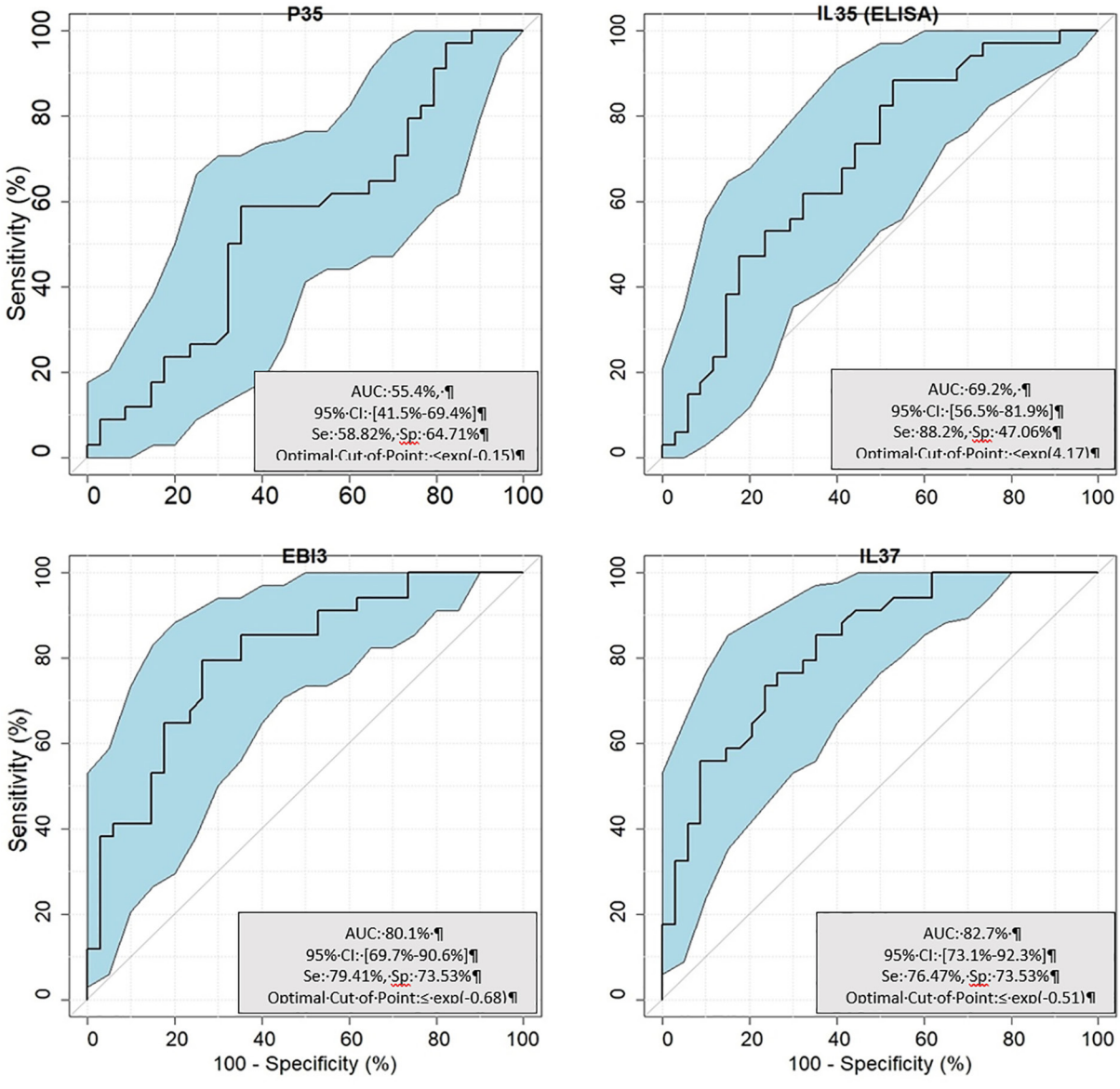

Fig. 2. ROC curve analysis for P35, Ebi3 and IL-37 transcripts as well as serum levels of IL-35 based on the results of the Bayesian regression model (Z=Z-Score, S*p*=Specificity, Se=Sensitivity, AUC=Area under curve, CI=Confidence interval)

Based on ELISA results, PD patients were found to have higher serum levels of IL-35 compared to the healthy control group (62±16 vs. 52±16 pg/µl). The mean IL35 in the control groups was 9.78 units lower than in patients (p=0.016). Stratified by gender, IL-35 serum concentrations were significantly higher in male patients (posterior Beta=-7.69, p=0.039), whereas no such difference was observed in female patients versus female controls (Table 4 and Fig. 1).

ROC Curves

ROC curve analysis identified optimal cutoff values of -0.15 for P35, -0.68 for Ebi3, -0.51 for IL-37, and 4.17 for IL-35 (ELISA).

Comparative evaluation of diagnostic performance revealed that that Ebi3 (AUC=80.1%, specificity=73.53% and sensitivity=79.41%) and IL-37 (AUC=82.7%, specificity=73.53% and sensitivity=76.47%) showed particularly strong potential as biomarkers for PD diagnostic panels (Fig. 2). However, further validation with larger sample sizes is required to confirm these findings.

Correlation Analysis

Pairwise correlation analysis revealed significant positive correlations between

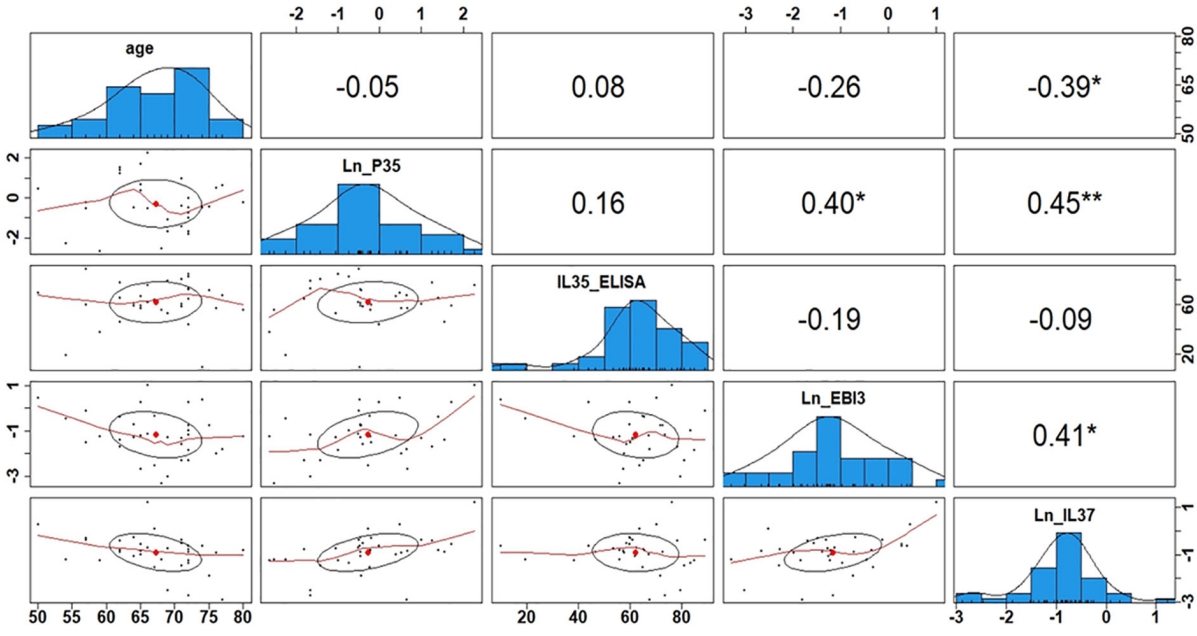

Fig. 3. Correlations between the expression levels of P35, Ebi3 and IL-37 genes, serum levels of IL-35 and age with each other based on the results of the Bayesian regression model. The correlation coefficients between the indices range from -1 to 0, signifying a negative correlation, and from 0 to +1, signifying a positive correlation. $_{*}p$=0.01-0.05, $_{**}p$=0.01-0.001, $_{***}p$<0.001

gene expression of P35 and Ebi3 (p=0.02, r=0.4), P35 and IL-37 (p=0.008, r=0.45), and Ebi3 with IL-37 (p=0.016, r=0.41) (Fig. 3). Additionally, we found a significant negative correlation between age and IL-37 gene expression (p=0.022, r=-0.39, Fig. 3).

DISCUSSION

In the present study, a significant decrease in IL-37 gene expression was observed in PD patients compared to the healthy control group. IL-37, as a key regulator of the immune system, has garnered the attention of researchers as a potential therapeutic agent for reducing inflammation. In this regard, Lonnemann and his colleagues demonstrated in 2022 that in hIL-37 transgenic mice (mice expressing human IL-37) crossed with an animal model of Alzheimer's disease, IL-37 was able to decrease the production of inflammatory cytokines and the activation of microglia. As a result, it improved the behavioral disorders in these mice (20). The anti-inflammatory effects of IL-37 have been well-documented in human studies. Treating human microglia obtained from the brains of healthy individuals with recombinant human IL-37 resulted in the suppression of IL-1β and CXCL8 gene expression stimulated by neurotensin (21). The mechanisms underlying the anti-inflammatory effects of IL-37 appear multifaceted. One of these mechanisms involves limiting cytokine production and inflammatory pathways. For example, since IL-18 levels are increased in PD patients compared to healthy individuals (22), IL-37 is believed to inhibit IL-18 activity and prevent inflammation by binding to IL-18Rα (23, 24). Moreover, induction of tolerogenic dendritic cells can be considered as another anti-inflammatory mechanism of IL-37 (25). Given the essential role of dendritic cells in PD, especially in presenting alpha-synuclein epitopes to T cells, IL-37 can slow down disease progression (26). In fact, IL-37 is a key regulator of the immune system with anti-inflammatory properties, and decreased expression of this cytokine could indicate a defect in the body's natural mechanisms to control inflammation. This reduction can be seen as one of the predisposing factors for the exacerbation of inflammatory processes and nerve damage.

IL-35, another anti-inflammatory cytokine from the IL-12 family, is a heterodimer composed of p35 and EBI3 subunits (27). IL-35 is mainly produced by regulatory T cells (Tregs) and regulatory B cells (Bregs) and suppresses the activity of effector T cells, while promoting the activity of Tregs and inhibiting the production of inflammatory cytokines (28). In our study, we specifically examined P35 and Ebi3 gene expression using qRT-PCR technique. Since these subunits are shared with other cytokines (P35 with IL-12 and Ebi3 with IL-27), we complemented our gene expression analysis with direct IL-35 serum quantification by ELISA to confirm the qRT-PCR results. Our findings demonstrated that gene expression of Ebi3 was significantly reduced in PD patients compared to the control group, while no significant difference was observed in P35 gene expression between the two groups. Interestingly, despite the decreased Ebi3 expression, we observed elevated IL-35 serum level in PD patients. This apparent discrepancy may be partially explained by the reported reduction of IL-27 (which shares the Ebi3 subunit) in PD patients. Kouchaki and coworkers found that decreased IL-27 serum level in PD patients significantly correlated with the disease progression (29). Several reasons could account for the observed inconsistency in IL-35 gene expression and serum protein levels. It should be noted that gene expression alterations do not always correlate with changes in the corresponding protein levels. There is a lengthy process from mRNA transcription to the formation of a mature and functionally active protein, where post-transcriptional modifications and translation regulators play crucial roles. Additionally, there are instances in which mRNA expression is decreased while protein stability is increased, as seen in the study conducted by Li and colleagues. These researchers found that despite the increase in stability of the alpha-synuclein protein in mature neurons, its mRNA expression decreased (30). This suggests that a similar mechanism may have led to increased stability of IL-35 and consequently higher protein levels in the sera of PD patients while gene expression decreased. An important consideration is that our gene expression analysis was limited to peripheral blood leukocytes. Elevated serum IL-35 levels observed in PD patients may originate from alternative cellular sources. Notably, Dzamko et al. demonstrated that neuronal and glial cells represent major contributors to cytokine production in PD (31). This suggests that central nervous system-derived cells could be primarily responsible for the increased serum level of IL-35 in our study.

All enrolled PD patients were receiving levodopa. Levodopa is the primary treatment for PD, as it is converted to dopamine by the enzyme aromatic L-amino acid decarboxylase. Increasing dopamine levels in the brain alleviate symptoms of PD such as tremors, muscle stiffness, and reduced movement (32). However, It has been shown that long-term use of levodopa can cause side effects such as neurotoxicity and pro-inflammatory responses in patients with PD (33). However, recent findings by Chen *et al.* demonstrate that levodopa injection in animal models of PD could improve behavioral disorders by inhibiting the activation of the NLRP3 inflammasome and reducing the inflammatory cytokines IL-1β and IL-18 (34). On the other hand, IL-1β and IL-18 are inflammatory triggers that lead to the production of IL-37 (35). Considering the role of levodopa in reducing these cytokines, one potential explanation for the decreased expression of IL-37 could be linked to patients' consumption of levodopa. Therefore, the duration of levodopa consumption, a key variable in our research, may have influenced the inflammatory response dynamics. This variable could be seen as a potential factor contributing to the lack of significant differences in P35gene expression between the study groups.

Our revealed gender-specific patterns as decreased expression of Ebi3 and increased IL-35 serum levels observed exclusively in male PD patients. In contrast, IL-37 reduction in PD patients occurred in both genders, indicating gender-independent expression. Additionally, we identified an age-associated decline in IL-37 expression among patients. The observed gender- and age-dependent expression patterns may reflect hormonal influences and aging-associated epigenetic modifications on immune gene regulations. Sex-specific expression likely

stems from distinct hormonal environments -testosterone's immunosuppressive and regulatory functions in males, versus estrogen's immune-enhancing properties in females (36, 37). IL-37 gene expression showed a significant negative correlation with age, supporting epigenetic involvement in age-related immune changes. These epigenetic alterations, such as chromatin remodeling, DNA methylation shifts, histone modifications, and deregulation of microRNA expression, are well-documented hallmarks of the aging process (38, 39). Therefore, decreased IL-37 expression in older individuals may contribute to inflammaging, the chronic low-grade inflammation linked age-related diseases. Additionally, borderline significant correlations (for example, $p=0.087$ for serum IL-35 in females) may reflect limited sample size, warranting larger studies for definitive conclusions. While not statistically significant, higher serum IL-35 in female PD patients in comparison to female controls (Table 4) may relate to levodopa treatment, consistent with our IL-37 discussion. Notably, levodopa appears to upregulate IL-35 more prominently in males likely due to sex-specific hormonal influences.

Correlation analysis revealed significant positive associations between P35-Ebi3, P35-IL-37, and Ebi3-IL-37. The P35-Ebi3 correlation is expected as these subunits together form IL-35, though, their participation in the structure of other cytokines (like P35 in IL-12 and IL-27) must be considered. According to ROC analysis, Ebi3 and IL-37 genes expression, demonstrating high sensitivity and specificity, may serve as potential diagnostic biomarkers for Parkinson's disease.

This study has limitations, including small sample size, particularly in gender subgroups and the use of peripheral samples rather than CNS-derived tissues which would provide a more accurate representation of regional immune activity and gene expression alterations. However, key challenges limit CNS sample collection in PD research: brain specimens from PD patients are only accessible post-mortem due to ethical barriers. On the other hand, the collection of cerebrospinal fluid (CSF) samples via lumbar puncture can cause discomfort, infection risks, and neurological complications. A third limitation is that IL-37 expression was only measured at the mRNA level. Future studies should analyze protein expression in larger CNS samples to clarify the pathophysiological significance of IL-35 and IL-37 in PD

CONCLUSION

In conclusion, our study showed a higher serum protein level of IL-35 in PD patients compared to healthy control group while the gene expression levels of IL-37 and IL-35 subunit Ebi-3 were significantly reduced. Alterations in the expression of these cytokines are suggested to partly contribute to the immune dysregulation in this disease.

ACKNOWLEDGEMENT

This manuscript has been extracted from the MSc thesis of Fariba Akbari Gavabari at Hamadan University of Medical Sciences, Hamadan, Iran. It has received financial support from Hamadan Medical University (Grant number: 140209288717). It is also worth noting that some parts of our experiments were conducted in the Core Facility Laboratory at Hamadan University of Medical Sciences.

AUTHORS' CONTRIBUTION

FAG and MC conducted the experiments and wrote the manuscript. ETG analyzed the data. MME and SA supervised the study. MRP reviewed the manuscript. MM served as the clinical consultant and assessed patients for inclusion in the study. AB collected the samples. SS provided assistance as a scientific advisor. All authors have approved the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Simon DK, Tanner CM, Brundin P. Parkinson Disease Epidemiology, Pathology, Genetics, and Pathophysiology. Clin Geriatr Med. 2020 Feb;36(1):1-12. PubMed PMID: 31733690. Pubmed Central PMCID: PMC6905381. Epub 2019/11/18. eng.
2. Harms AS, Ferreira SA, Romero-Ramos M. Periphery and brain, innate and adaptive immunity in Parkinson's disease. Acta Neuropathol. 2021 Apr;141(4):527-45. PubMed PMID: 33555429. Pubmed Central PMCID: PMC7952334. Epub 2021/02/09. eng.
3. Marogianni C, Sokratous M, Dardiotis E, Hadjigeorgiou GM, Bogdanos D, Xiromerisiou G. Neurodegeneration and Inflammation-An Interesting Interplay in Parkinson's Disease. Int J Mol Sci. 2020 Nov 10;21(22). PubMed PMID: 33182554. Pubmed Central PMCID: PMC7697354. Epub 2020/11/14. eng.
4. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. Nat Rev Immunol. 2022 Nov;22(11):657-73. PubMed PMID: 35246670. Pubmed Central PMCID: PMC8895080 describing the dominant-negative TNFs and is a consultant to and has stock ownership in Xencor and Inmune Bio, which has licensed Xpro1595 (pegipanermin) for neurological indications. All other authors declare no competing interests. Epub 2022/03/06. eng.
5. Williams GP, Schonhoff AM, Sette A, Lindestam Arlehamn CS. Central and Peripheral Inflammation: Connecting the Immune Responses of Parkinson's Disease. J Parkinsons Dis. 2022;12(s1):S129-s36. PubMed PMID: 35754290. Pubmed Central PMCID: PMC9535591. Epub 2022/06/28. eng.
6. Zimmermann M, Brockmann K. Blood and Cerebrospinal Fluid Biomarkers of Inflammation in Parkinson's Disease. J Parkinsons Dis. 2022;12(s1):S183-s200. PubMed PMID: 35661021. Pubmed Central PMCID: PMC9535573. Epub 2022/06/07. eng.
7. Ye C, Yano H, Workman CJ, Vignali DAA. Interleukin-35: Structure, Function and Its Impact on Immune-Related Diseases. J Interferon Cytokine Res. 2021 Nov;41(11):391-406. PubMed PMID: 34788131. Pubmed Central PMCID: PMC8820099. Epub 2021/11/18.
8. Zhang J, Zhang Y, Wang Q, Li C, Deng H, Si C, et al. Interleukin-35 in immune-related diseases: protection or destruction. Immunology. 2019 May;157(1):13-20. PubMed PMID: 30681737. Pubmed Central PMCID: PMC6459776. Epub 2019/01/27.
9. Su LC, Liu XY, Huang AF, Xu WD. Emerging role of IL-35 in inflammatory autoimmune diseases. Autoimmun Rev. 2018 Jul;17(7):665-73. PubMed PMID: 29729445. Epub 2018/05/08.
10. Song M, Ma X. The Immunobiology of Interleukin-35 and Its Regulation and Gene Expression. Advances in experimental medicine and biology. 2016;941:213-25. PubMed PMID: 27734415. Epub 2016/10/14. eng.
11. Niedbala W, Wei XQ, Cai B, Hueber AJ, Leung BP, McInnes IB, et al. IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. European journal of immunology. 2007 Nov;37(11):3021-9. PubMed PMID: 17874423. Epub 2007/09/18. eng.
12. Teymouri M, Pirro M, Fallarino F, Gargaro M, Sahebkar A. IL-35, a hallmark of immune-regulation in cancer progression, chronic infections and inflammatory diseases. Int J Cancer. 2018 Nov 1;143(9):2105-15. PubMed PMID: 29574719. Epub 2018/03/27.
13. Su Z, Tao X. Current Understanding of IL-37 in Human Health and Disease. Frontiers in immunology. 2021;12:696605. PubMed PMID: 34248996. Pubmed Central PMCID: PMC8267878. Epub 2021/07/13. eng.
14. Li S, Amo-Aparicio J, Neff CP, Tengesdal IW, Azam T, Palmer BE, et al. Role for nuclear interleukin-37 in the suppression of innate immunity. Proceedings of the National Academy of Sciences of the United States of America. 2019 Mar 5;116(10):4456-61. PubMed PMID: 30792349. Pubmed Central PMCID: PMC6410848. Epub 2019/02/23. eng.
15. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. Nature immunology. 2010 Nov;11(11):1014-22. PubMed PMID: 20935647. Pubmed Central PMCID: PMC3537119. Epub 2010/10/12. eng.
16. Li X, Yan B, Du J, Xu S, Liu L, Pan C, et al. Recent Advances in Progresses and Prospects of IL-37 in Central Nervous System Diseases. Brain Sci. 2022 May 31;12(6). PubMed PMID: 35741608. Pubmed Central PMCID: PMC9221119. Epub 2022/06/25. eng.
17. Moretti S, Bozza S, Oikonomou V, Renga G, Casagrande A, Iannitti RG, et al. IL-37 inhibits inflammasome activation and disease severity in murine aspergillosis. PLoS pathogens. 2014 Nov;10(11):e1004462. PubMed PMID: 25375146. Pubmed Central PMCID: PMC4223056. Epub 2014/11/07. eng.
18. Jia H, Liu J, Han B. Reviews of Interleukin-37: Functions, Receptors, and Roles in Diseases. Biomed Res Int. 2018;2018:3058640. PubMed

PMID: 29805973. Pubmed Central PMCID: PMC5899839. Epub 2018/05/29. eng.
19. Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. Applied linear statistical models. 4th ed ed. Chicago: Irwin; 1996.
20. Lonnemann N, Hosseini S, Ohm M, Geffers R, Hiller K, Dinarello CA, et al. IL-37 expression reduces acute and chronic neuroinflammation and rescues cognitive impairment in an Alzheimer's disease mouse model. eLife. 2022 Aug 30;11. PubMed PMID: 36040311. Pubmed Central PMCID: PMC9481244. Epub 2022/08/31. eng.
21. Tsilioni I, Patel AB, Pantazopoulos H, Berretta S, Conti P, Leeman SE, et al. IL-37 is increased in brains of children with autism spectrum disorder and inhibits human microglia stimulated by neurotensin. Proceedings of the National Academy of Sciences of the United States of America. 2019 Oct 22;116(43):21659-65. PubMed PMID: 31591201. Pubmed Central PMCID: PMC6815178. Epub 2019/10/09. eng.
22. Zhang P, Shao XY, Qi GJ, Chen Q, Bu LL, Chen LJ, et al. Cdk5-Dependent Activation of Neuronal Inflammasomes in Parkinson's Disease. Movement disorders : official journal of the Movement Disorder Society. 2016 Mar;31(3):366-76. PubMed PMID: 26853432. Epub 2016/02/09. eng.
23. Bufler P, Azam T, Gamboni-Robertson F, Reznikov LL, Kumar S, Dinarello CA, et al. A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. Proceedings of the National Academy of Sciences of the United States of America. 2002 Oct 15;99(21):13723-8. PubMed PMID: 12381835. Pubmed Central PMCID: PMC129755. Epub 2002/10/17. eng.
24. Kumar S, Hanning CR, Brigham-Burke MR, Rieman DJ, Lehr R, Khandekar S, et al. Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-gamma production. Cytokine. 2002 Apr 21;18(2):61-71. PubMed PMID: 12096920. Epub 2002/07/05. eng.
25. Luo Y, Cai X, Liu S, Wang S, Nold-Petry CA, Nold MF, et al. Suppression of antigen-specific adaptive immunity by IL-37 via induction of tolerogenic dendritic cells. Proceedings of the National Academy of Sciences of the United States of America. 2014 Oct 21;111(42):15178-83. PubMed PMID: 25294929. Pubmed Central PMCID: PMC4210310. Epub 2014/10/09. eng.
26. Park SY, Yang H, Kim S, Yang J, Go H, Bae H. Alpha-Synuclein-Specific Regulatory T Cells Ameliorate Parkinson's Disease Progression in Mice. Int J Mol Sci. 2023 Oct 16;24(20). PubMed PMID: 37894917. Pubmed Central PMCID: PMC10607030. Epub 2023/10/28. eng.
27. Choi JK, Egwuagu CE. Interleukin 35 Regulatory B Cells. J Mol Biol. 2021 Jan 8;433(1):166607. PubMed PMID: 32755620. Pubmed Central PMCID: PMC7779660. Epub 2020/08/07.
28. Bello RO, Chin VK, Abd Rachman Isnadi MF, Abd Majid R, Atmadini Abdullah M, Lee TY, et al. The Role, Involvement and Function(s) of Interleukin-35 and Interleukin-37 in Disease Pathogenesis. Int J Mol Sci. 2018 Apr 11;19(4). PubMed PMID: 29641433. Pubmed Central PMCID: PMC5979316. Epub 2018/04/12.
29. Kouchaki E, Kakhaki RD, Tamtaji OR, Dadgostar E, Behnam M, Nikoueinejad H, et al. Increased serum levels of TNF-α and decreased serum levels of IL-27 in patients with Parkinson disease and their correlation with disease severity. Clinical neurology and neurosurgery. 2018 Mar;166:76-9. PubMed PMID: 29408778. Epub 2018/02/07. eng.
30. Li W, Lesuisse C, Xu Y, Troncoso JC, Price DL, Lee MK. Stabilization of alpha-synuclein protein with aging and familial parkinson's disease-linked A53T mutation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004 Aug 18;24(33):7400-9. PubMed PMID: 15317865. Pubmed Central PMCID: PMC6729772. Epub 2004/08/20. eng.
31. Dzamko N. Cytokine activity in Parkinson's disease. Neuronal signaling. 2023 Dec;7(4):Ns20220063. PubMed PMID: 38059210. Pubmed Central PMCID: PMC10695743. Epub 2023/12/07. eng.
32. Nishijima H, Tomiyama M. What Mechanisms Are Responsible for the Reuptake of Levodopa-Derived Dopamine in Parkinsonian Striatum? Frontiers in neuroscience. 2016;10:575. PubMed PMID: 28018168. Pubmed Central PMCID: PMC5156842. Epub 2016/12/27. eng.
33. Dorszewska J, Prendecki M, Lianeri M, Kozubski W. Molecular Effects of L-dopa Therapy in Parkinson's Disease. Current genomics. 2014 Feb;15(1):11-7. PubMed PMID: 24653659. Pubmed Central PMCID: PMC3958954. Epub 2014/03/22. eng.
34. Chen X, Wang Z, Yang W, Fu Y. Levodopa Improves Behavioral Deficits of Mice with Parkinson's Disease Symptoms via Curbing NLRP3 Inflammasome Activation and Enhancing Tyrosine Hydroxylase Levels in the Striatum and Substantia Nigra. Journal of integrative neuroscience. 2024 Jan 10;23(1):2. PubMed PMID: 38287845. Epub 2024/01/30. eng.
35. Santarelli DM, Vincent FB, Rudloff I, Nold-Petry CA, Nold MF, Russo MA. Circulating Interleukin-37 Levels in Healthy Adult Humans - Establishing a Reference Range. Frontiers in immunology. 2021;12:708425. PubMed PMID: 34367169. Pubmed Central PMCID: PMC8343013. Epub 2021/08/10. eng.

36. Sciarra F, Campolo F, Franceschini E, Carlomagno F, Venneri MA. Gender-Specific Impact of Sex Hormones on the Immune System. Int J Mol Sci. 2023;24(7).
37. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Human reproduction update. 2005;11(4):411-23.
38. Saul D, Kosinsky RL. Epigenetics of Aging and Aging-Associated Diseases. Int J Mol Sci. 2021;22(1).
39. Jasiulionis MG. Abnormal Epigenetic Regulation of Immune System during Aging. Frontiers in immunology. 2018;9:197.