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Concomitant Expression of IL-6 and TGF-β Cytokines and their Receptors in Peripheral Blood of Patients with Multiple Sclerosis: The Effects of INFβ Drugs

Parisa Maleki[1], Bahareh Abd-Nikfarjam[2], Azam Jamshidian[3]*

[1]Department of Microbiology and Immunology, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran; [2]Department of Immunology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran; [3]Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran

ABSTRACT

Background: Concomitant signals from IL-6 and TGF-β have a central role in the Th17 cells development and differentiation, and these cells are the main promoters of demyelinating inflammation in the central nervous system (CNS) resulting in multiple sclerosis (MS).

Objectives: To evaluate the simultaneous IL-6 and TGF-β gene and their receptor protein expression in patients with Relapsing-Remitting (RR)-MS.

Methods: IL-6 and TGF-β mRNA and their receptor expression on the surface of CD4+T cells were evaluated using real-time PCR (RT-PCR) and flow cytometry, respectively.

Results: The IL-6 mRNA expression in patients with RRMS was significantly higher than in the controls (P=0.019). When patients who did not receive any other treatment were compared with the controls, the significant difference was substantial (P=0.006). The TGF-β mRNA expression in patients was lower than in the controls (P=0.03). However, in patients receiving IFNβ, it increased compared with the other patients (P=0.036). There was no difference in cytokine receptor expression between patients and the control group.

Conclusion: Our data conclude an increase and decrease in mRNA expression levels of IL-6 and TGF-β in patients with RRMS, respectively. Moreover, there were no significant differences in receptor expression of either cytokines. Based on our data the balance of TGF and IL-6 appears to have a positive impact on the disease control.

Keywords: IL6 Receptor, TGFβ Receptor, Th17

*Corresponding author:
Azam Jamshidian,
Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran
Email: azamjamshidian@gmail.com

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INTRODUCTION

Th17 cells play a central role in mediating autoimmune and inflammatory diseases such as multiple sclerosis (1, 2). Furthermore, the Th17 cells increase not only in the brain but also in the peripheral blood of RRMS patients (1, 3).

IL-6 is a pivotal cytokine in the induction of the Th17 phenotype in $CD4^+$T cells, and dysregulations in IL-6 receptors are correlated with the upregulation of IL-17A-related signaling in these cells (4). Moreover, IL-6 inhibits $CD4^+$ T cell differentiation in regulatory T (Treg) cells, leading to changes in the Th17/Treg balance (5). Also, IL-6 in the cerebrospinal fluid directly affects disease activity in multiple sclerosis (6).

TGF-β, in addition to IL-6, IL23, and IL1, is required for the development of Th17 cells (7). Furthermore, this cytokine has received a significant attention as a major immune regulatory factor in RRMS and other autoimmune diseases (8). It was realized that TGF-β obstacles the production of IL-2 as well as the proliferation of T cells (9). Moreover, it was found that TGF-β can modulate immune cell functions both positively and negatively (8). The lack of TGF-β signaling results in an uncontrolled T cell differentiation, the onset of autoimmune diseases, and systemic inflammation (9). Several studies reported that the treatment of experimental autoimmune encephalomyelitis (EAE) with TGF-β resulted in alleviated neurological symptoms (9).

IFNβ was discovered in 1957 as a protective cytokine that reduces inflammation, currently deemed a neuroprotective drug (10). Several studies in EAE models also illustrated that IFNβ can inhibit demyelination caused by the immune system and it practically can improve brain atrophy and lesion in RRMS patients (11). Regarding these findings, IFNβ was the first approved drug for RRMS patients, ameliorating the severity and repetition of relapses and disease progression. IFNβ not only acts on myeloid cells but also inhibits cytokine production. Thus, exogenous IFNβ exerts useful influences on the immune system modulation (10). IFNβ also downregulates several inflammatory pathways leading to the induction of Th17 cells (1).

Regarding the significant role of IL-6 and TGF-β in Th17 cell differentiation, in order to better understand the importance of Treg/Th17 balance in MS pathogenesis, we evaluated the simultaneous changes in mRNA expression of these cytokines and protein expression of their receptors on the surface of $CD4^+$ T cells in RRMS patients under the effects of IFN-β, other different disease modifying drugs, and patients who consume no immunosuppressive drug, compared with the healthy controls.

MATERIALS AND METHODS

RRMS Patients and the Healthy Controls

Forty patients with RRMS diagnosed based on McDonald MS diagnostic criteria (12), were admitted to the Neurology Ward, Kashani Hospital, Shahrekord, Iran from January to May 2018, and as controls, forty age, gender, and ethnicity-matched healthy individuals took part in this study . Fourteen patients used IFN-β drugs and fourteen patients used other-drug, twelve more cases did not consume any immunomodulating drug for at least 3 months. All the patients were in the remission phase, and their last high-dose corticosteroid medication had been at least three months prior (steroid pulse therapy). Patients with infectious diseases, other autoimmune diseases, and significant neurological disorders along with MS, were excluded from the study. Kurtzke's score, Expanded Disability Status Scale (EDSS), was used for evaluating the clinical severity of the disease. Demographic data and clinical characteristics of study groups are shown in Table 1. All survey respondents signed informed consent forms. The local Research Ethics Committee at Shahrekord University of Medical Sciences in Shahrekord, Iran, granted ethical approval (Approval code: IR.SKUMS.REC1398.48).

Flow Cytometry

Ficoll-Hypaque density gradient method was used to isolate the PBMCs from fresh whole blood of the patients and the controls. 1×10^5 PBMCs within 2 hours of separation

Table 1. Demographic and Clinical characteristics of the RRMS patients and healthy individual control groups

	Patient Group	Control Group
Mean age±SD, year (range)	32.58±11.26 (20-55)	33.27±12.42 (21-57)
Sex	Female (35)-Male (3)	Female (38)-Male (2)
EDSS	1.49±1.14	
Drugs	IFN-β (Cinnovex, Ziferon, Actofron)	-
	Other-drugs (Teriflunomide&Copaxone)	-
	Non-drug	-
Mean disease duration±SD, months	69.58±27.2	-
Mean age at onset of disease	28.3±4.28	-
Mean number of relapses in the previous year	2.2±1.7	-

were stained with conjugated antibodies against 3 markers, CD4, TGF-β Receptor, and IL-6 Receptor. The used antibodies were anti-human CD4 conjugated with peridinin chlorophyll protein (PerCP)-Cy5.5, anti-human TGF-β Receptor conjugated with fluorescein isothiocyanate (FITC), and anti-human IL-6 Receptor conjugated with phycoerythrin (PE) (R&D Systems, Abingdon, Oxfordshire, UK). Samples were incubated at 4 °C for 20 min, and then at room temperature for 20 min

For setting the exact gates and quadrants and determining non-specific binding of antibodies and background fluorescence emittance, 3 isotype-matched control antibodies (all from BD Biosciences, NSW, Australia) were used in a parallel tube for each sample. For the elimination of unbounded conjugated antibodies, the cells were washed twice with PBS.

Partec CyFlow Space flow cytometer instrument (Partec, Münster, Germany) was used to carry out the tree-color Flow cytometry analysis. In each tube, 10^5 events were acquired, and the gating was down based on their side and forward light scatter characteristics.

To analyze the acquired data, Flomax Data Acquisition and Analysis Software (Partec, Germany), and Flowjo 7.6.1 analysis software were used.

RNA extraction, and Real Time-PCR

Total RNA extraction from PBMCs was down by Trizol reagent (Invitrogen). The purification and quantity of the extracted RNA were measured using a Thermo Scientific NanoDrop Spectrophotometer at 260/280 and 260/230 ratios.

Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was used and the cDNA was synthesized from one microgram of total RNA according to the manufacturer's protocol. SYBRGreen/RoxqPCR Mastermix ((TAKARA, Japan) and the quantitative Real-time PCR Detection System (Rotor-Gene RG-300, Corbett Research, Sydney, Australia) were used for carrying out Real-time PCR.

Each reaction volume was 20 µL, with 200 µM forward and reverse primers, 10 µL SYBR Green, and 1.5 µL cDNA. PCR cycling program was used for 38 cycles as follows: 15 seconds for denaturation at 95°C, 20 seconds for annealing at 61°C, and 25 seconds for an extension at 72°C. The reference gene for normalizing the relative gene expression was GAPDH. The target gene expression value was calculated by Delta-Delta Ct ($2^{-\Delta\Delta Cq}$) method. Specific primers used in Real-Time PCR are indicated in Table 2 by their sequences.

Statistical Analysis

The software Prism 5 (GraphPad Software,

Table 2. The sequence of specific primers for RT-PCR

Primer	Primer sequence (5′ to 3′)	Length of product
IL-6 forward	5-AATTCGGTACATCCTCGACGG-3	98 bp
IL-6 reverse	5-GGTTGTTTTCTGCCAGTGCC-3	
TGF-β forward	5-TACCTGAACCCGTGTTGCTCT-3	110 bp
TGF-β revers	5-ATCGCCAGGAATTGTTGCTG-3	
GAPDH forward	5-CCTCTGACTTCAACAGCGACAC-3	117 bp
GAPDH reverse	5-TGGTCCAGGGGTCTTACTCC-3	

La Jolla, Calif) was used to perform statistical analysis. The data were shown as mean±SD, had a normal distribution and was analyzed using a parametric sample t-test and one-way ANOVA (post hoc test: Tukey multiple comparisons). P values<0.05 were reported as significant.

RESULTS

Clinical data and demographic characteristics of the study groups are demonstrated in Table 1. The IL-6 mRNA expression in the PBMCs of MS patients significantly increased compared with the healthy controls (Figure 1A). Moreover, the level of IL-6 mRNA expression in patients who did not use any drug was two times higher than in the healthy controls (P=0.006); however, there was no significant difference in IL-6 mRNA expression between the three patient groups (IFNβ users, other-drug users and patients with no-drug consumption). (P>0.05, Figure 1). Moreover, IL-6 mRNA expression positively correlated with the age in the patient groups (P=0.026, Table 3).

The TGF-β mRNA expression in the patients was lower than in the control group. However, the mRNA level of the TGF-β in the IFNβ-drug RRMS patients increased compared with other-drug and non-drug

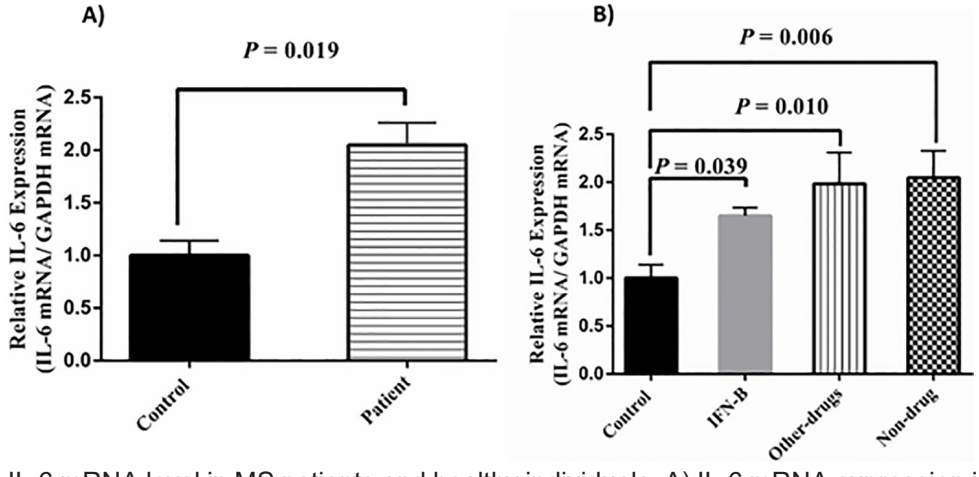

Figure 1. IL-6 mRNA level in MS patients and healthy individuals. A) IL-6 mRNA expression in patients was higher than control group. B) IL-6 mRNA expression in INFβ consuming MS patients was lower than other patient groups. The data from the real-time PCR for human IL-6 were normalized versus GAPDH.

Table 3. The Pearson correlation analysis

Variables		Correlation coefficient (r)	P value
IL6 mRNA	Patient Age	+0.423	0.026
TGFβ mRNA	Patient Age	-0.412	0.022
IL6 mRNA	TGFβ mRNA	-0.0291	0.031

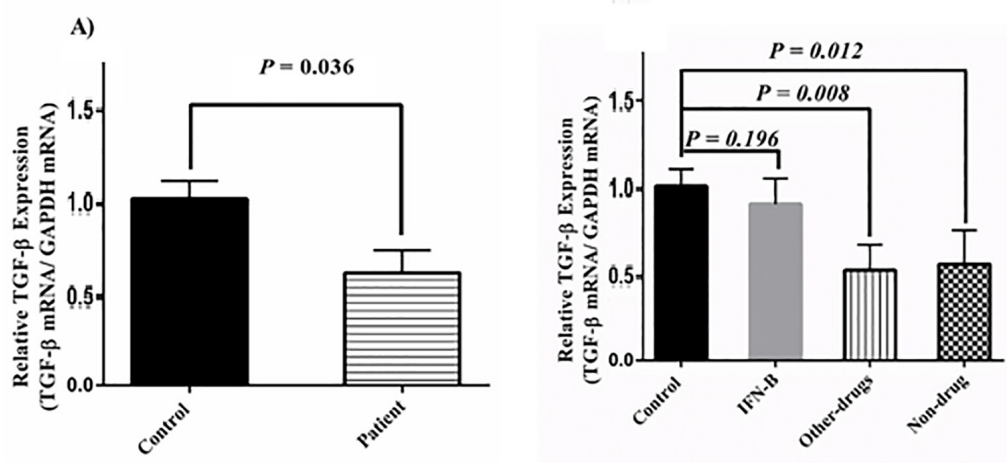

Figure 2. TGF-β mRNA level in the patients and control groups A) TGF-β mRNA expression in the patients was lower than the control group (P=0.036). B) The TGF-β gene was significantly overexpressed in PBMCs of INFβ consuming patient group compared to Non-drug and Other-drug groups.

Table 4. The percentage of T CD4⁺ Subtypes

T cells	Control	Patient	P value
$CD4^+$ $IL6R^+$	20.9%	18.64%	0.542
$CD4^+$ $TGF\beta R^+$	2.23%	2.59%	0.24
$TGF\beta R^+$ $IL6R^+$	7.79%	7.74%	0.968

RRMS patients (P=0.036, Figure 2A). Also, after doing ANOVA and post hock tests, it was shown that the significant difference is between the control group and other-drug and non-drug patient groups but not the IFNβ consuming group (Figure 2).

The phenotypic characterization of $CD4^+$T lymphocytes showed that the average percentage of IL-$6R^+$T cells, TGF-βR^+T cells, and IL-$6R^+$TGF-βR^+T cells was almost similar in RRMS patients and in the healthy control (P>0.05, Table 4 (Figure 3)). Interestingly, the percentage of $CD4^+$TGF-βR^+T cells in IFNβ-drugs patients (28.3%±4.06%), was higher than in other-drug (14.3%±5.659%), and non-drug patients (15.52%±2.204%), (P=0.011 and *P*=0.043, respectively) (Figure 4). The Pearson Correlation analysis showed a negative relation between TGF-β mRNA expression, the disease duration and IL-6 mRNA expression (Table 3). Also, the duration of the disease in patients significantly correlated with the percentage of $CD4^+$TGF-βR^+IL-$6R^+$ T cells (P=0.018, r=+0.427, Figure 5).

DISCUSSION

In the present study, we investigated the expression of IL-6 and TGF-β cytokines, and their receptors in PBMCs of RRMS patients in the remission phase and the healthy individuals. The RRMS patients studied were embedded in three groups based on the type of immunosuppressive drugs they were taking: IFNβ-drugs, other-drug, as well as non-drug.

The results of our study showed a significant increase in IL-6 mRNA expression in PBMCs of MS patients compared with the healthy individuals. However, the level of IL-6 mRNA decreased in IFNβ-drug consuming patients compared with other MS patients, which means that IFNβ reduces IL-6 mRNA expression, thus this research backs up prior studies. Previous experiments have shown that IFNβ reduced the production of IL17 by $CD4^+$T cells under stimulation with IL-6 and TGF-β (13). Likewise, IFNβ decreased IL17 in effector and memory cells which were induced with IL-6 and TGF-β (1, 13).

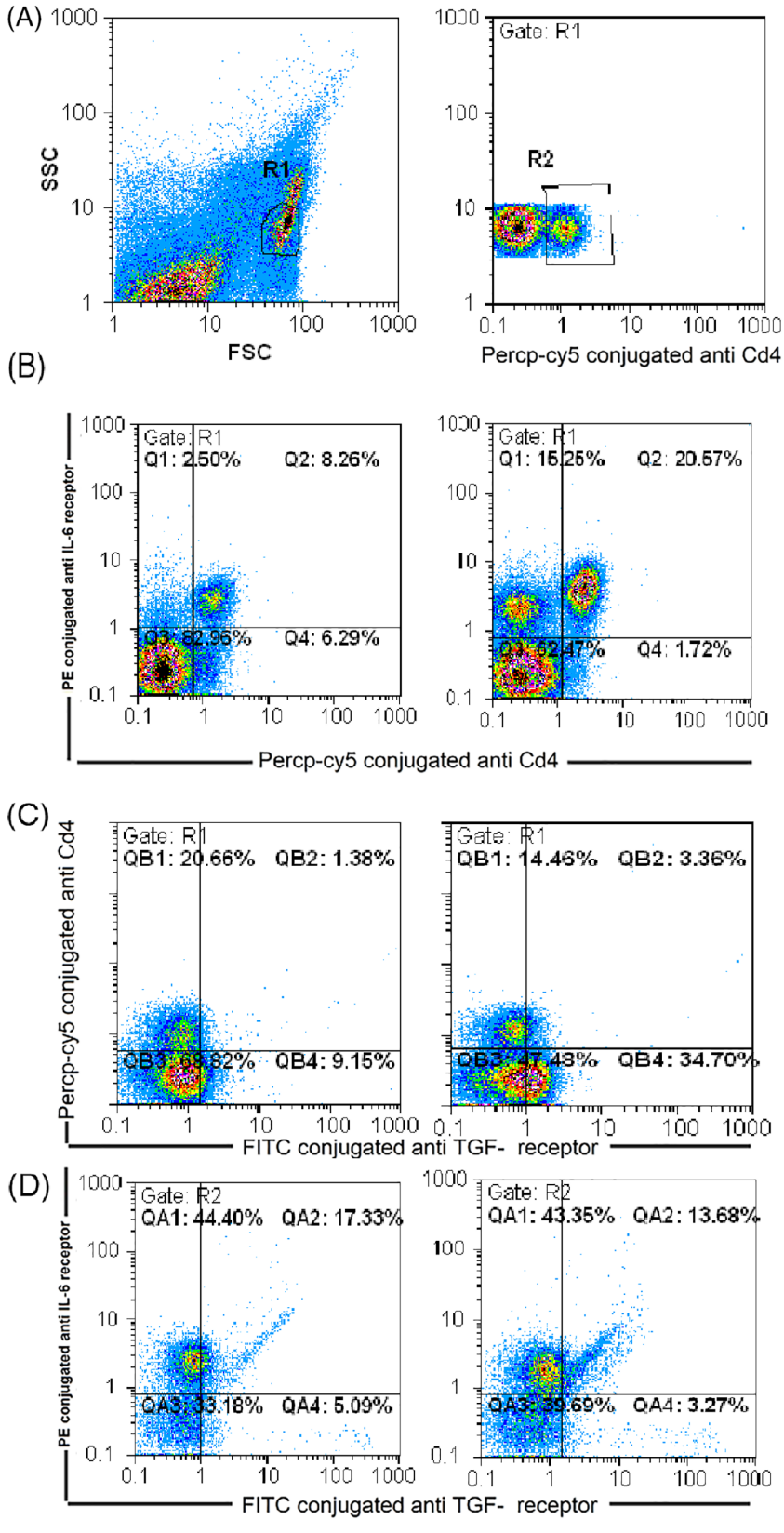

Figure 3. TGF-βR and IL-6R expression on lymphocytes of RR MS patients and healthy controls. A, Gating of the lymphocyte population was done based on side and forward light scattering (SSC and FSC) properties (Left), then the CD4+ lymphocyte population was gated (Right). B-D, Representative dot plots of CD4+IL-6R+, CD4+TGF-βR+ and CD4+IL-6R+TGF-βR+ Lymphocytes in patient and control groups. In each quadrant, numbers represent the percentage of the related population

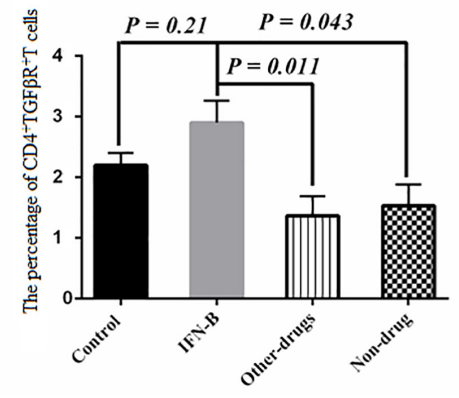

Figure 4. The percentage of $CD4^+TGF\text{-}\beta R^+$ T cells in IFNβ-drugs, Other-drugs and Non-drug consuming MS patient groups was 2.83%±0.40%, 1.43%±0.56% and 1.55%±0.22%, respectively.

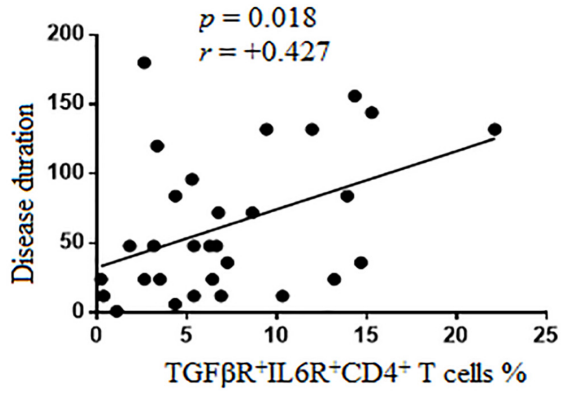

Figure 5. There was a positive correlation between disease duration and $CD4^+TGF\text{-}\beta R^+IL\text{-}6R^+$T cell percentage

This illustrates that IFNβ attenuates the early and late stages of Th17 cell differentiation. Furthermore, previous studies have shown that IFNβ downregulates IL-17 expression directly through the STAT1 activation and STAT3 suppression (14). But conversely, some recent in-vitro and mouse studies have shown that IFN-β has various effects on separate stages of TH17 development (15), and even raises the production of IL-6 by B-cells and deteriorates TH17-mediated diseases (16).

We also detected a considerable decrease in the TGF-β mRNA level in other-drug and non-drug MS patient groups compared with the control and INFβ MS patients. These results showed that INFβ therapy may cause a correction in the pathologic decrease in TGF-β gene expression in MS patients and its achievement in the normal levels. Consistent with these results, prior studies have shown that increasing TGF-β signals in MS patients' T cells promotes improvements in the symptoms of the condition (17). In a previous study, we found that the expression of TGF-β mRNA increased after therapeutic plasma exchange (TPE) in RRMS patients in the relapse phase (18). These findings are notable since both TPE and IFNβ treatments improve the symptoms of RRMS disease by increasing the expression of TGF-β mRNA in the relapse and remission phases, respectively (19, 20). Moreover, IFNβ prominently alters the phenotype of memory Th cells to Tregs which reduces the clinical parameters including EDSS, and the repetition of the relapses (17).

Prior research has also revealed that the frequency of Tregs declines in MS disease, and Treg/Th17 cells balance is impaired in the patients' blood (21). Furthermore, in the present study, the disease duration is shown to be directly correlated with the percentage of $CD4^+TGF\text{-}\beta R^+IL\text{-}6R^+$ T cells. As a result, as the duration of the sickness increases, so does the number of these cells. In addition, in our patient group, the IL-6 and TGF-β mRNA expressions were shown to be respectively in positive and negative correlation with the patients' age. In the relevant studies, the relationship between IL-6 and the age of MS patients has been a point of contention (6, 22, 23).

Previous studies have demonstrated that the concurrent effects of IL-6 and TGF-β cytokines are involved in the differentiation of naive $CD4^+$T cells to Th17 cells (7) and the dysregulation of Th17/Treg balance participates in the pathogenesis of MS disease (21). TGF-β plays a key part in the differentiation of Tregs, hence the suppression of inflammation and autoimmune diseases (7).

As mentioned before, Th17 cell development from $CD4^+$T cells requires simultaneous signaling of both IL-6 and TGF-β cytokines. Thus, IL-6R and TGF-βR are important mediators in Th17 cell differentiation and pathogenesis of MS disease (24). The suppression of Th17 cells or modifying the Th17/Treg balance has been suggested as a

beneficial strategy to control the neuroimmune symptoms in MS patients (25).

Assuming the fact that IL-6 and TGF-β systemic regulation are probably involved in MS pathogenesis, the observed decrease in the mRNA level of TGF-β in other-drug and non-drug patient groups compared with INFβ consuming patient and the control groups seems important in the results of the present study. Several studies have documented the increase of Th17 cells in CNS lesions of MS patients, suggesting an essential role of Th17 cells in the immunopathogenesis of MS disease (1). IFN-β can directly suppress Th17 cell differentiation (26). Moreover, IFNβ downregulates several inflammatory pathways involved in the induction of the Th17 phenotype in naïve $CD4^+$T cells (27).

It was established by the findings of our inquiry that the proportion of $CD4^+IL\text{-}6R^+$T cells and $CD4^+TGF\text{-}\beta R^+$T cells were similar in both the patients and the control groups. However, in the INFβ consuming group, the $CD4^+TGF\text{-}\beta R^+$ T cell frequency was higher than in the other-drug and non-drug groups. A recent study in this regard has confirmed that both Th17 and Treg fate lines entail T cell receptor (TCR) and TGF-βR signals, and polarization to Th17 phenotype need further IL-6R signals. The high-resolution phosphoproteomic technique has helped to identify the synergistic and preservative interactions between TCR, TGF-βR, and IL-6R which delineates kinase signaling grids to diversely adjust main events throughout the initial phases of Treg against Th17 orientation (28).

Overall, these observations confirm the importance of TGF-β and IL-6 cytokines and their receptors as therapeutic targets in MS treatment. The restoration of the balance between IL-6 and TGF-β and their receptors' expression may be a precise description of the beneficial therapeutic effects of IFNβ-drugs.

CONCLUSION

In RR-MS patients, the expression levels of TGF-β and IL-6 cytokines, in addition to the TGF-β receptor on the surface of CD4+ T cells, are different from the healthy controls. INFβ drugs can affect the expression of TGF-β, IL-6 and their receptors and consequently change the phenotype of Th cells. It seems that the modification of the TGF-β and IL-6 balance have beneficial effects on the disease control and improvement. IL-6 and TGF- mRNA could also be used as biomarkers for MS diagnosis and monitoring, as well as assessing the efficacy of new medications and therapies.

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Conflict of Interest: None declared.

REFERENCES

1. Moser T, Akgün K, Proschmann U, Sellner J, Ziemssen T. The role of TH17 cells in multiple sclerosis: Therapeutic implications. Autoimmunity Reviews. 2020:102647.
2. Maciak K, Pietrasik S, Dziedzic A, Redlicka J, Saluk-Bijak J, Bijak M, et al. Th17-Related Cytokines as Potential Discriminatory Markers between Neuromyelitis Optica (Devic's Disease) and Multiple Sclerosis—A Review. International Journal of Molecular Sciences. 2021;22(16):8946.
3. Balasa R, Barcutean L, Balasa A, Motataianu A, Roman-Filip C, Manu D. The action of TH17 cells on blood brain barrier in multiple sclerosis and experimental autoimmune encephalomyelitis. Human immunology. 2020;81(5):237-43.
4. Nadeem A, Ahmad SF, Attia SM, Al-Ayadhi LY, Al-Harbi NO, Bakheet SA. Dysregulation in IL-6 receptors is associated with upregulated IL-17A related signaling in CD4+ T cells of children with autism. Progress in Neuro-Psychopharmacology

and Biological Psychiatry. 2020; 97:109783.
5. Hsieh W-C, Hsu T-S, Chang Y-J, Lai M-Z. IL-6 receptor blockade corrects defects of XIAP-deficient regulatory T cells. Nature communications. 2018;9(1):1-15.
6. Stampanoni Bassi M, Iezzi E, Drulovic J, Pekmezovic T, Gilio L, Furlan R, et al. IL-6 in the cerebrospinal fluid signals disease activity in multiple sclerosis. Frontiers in Cellular Neuroscience. 2020; 14:120.
7. Wu B, Zhang S, Guo Z, Bi Y, Zhou M, Li P, et al. The TGF-β superfamily cytokine Activin-A is induced during autoimmune neuroinflammation and drives pathogenic Th17 cell differentiation. Immunity. 2021;54(2):308-23. e6.
8. Sotiropoulos MG, Chitnis T. Opposing and potentially antagonistic effects of BMP and TGF-β in multiple sclerosis: The "Yin and Yang" of neuro-immune Signaling. Journal of neuroimmunology. 2020:577358.
9. Lee PW, Severin ME, Lovett-Racke AE. TGF-β regulation of encephalitogenic and regulatory T cells in multiple sclerosis. European journal of immunology. 2017;47(3):446-53.
10. Hauser SL, Cree BA. Treatment of multiple sclerosis: a review. The American Journal of Medicine. 2020.
11. Bross M, Hackett M, Bernitsas E. Approved and emerging disease modifying therapies on neurodegeneration in multiple sclerosis. International journal of molecular sciences. 2020;21(12):4312.
12. Carroll WM. 2017 McDonald MS diagnostic criteria: Evidence-based revisions. SAGE Publications Sage UK: London, England; 2018. p. 92-5.
13. Axtell RC, De Jong BA, Boniface K, Van Der Voort LF, Bhat R, De Sarno P, et al. T helper type 1 and 17 cells determine efficacy of interferon-β in multiple sclerosis and experimental encephalomyelitis. Nature medicine. 2010;16(4):406-12.
14. Tsai M-H, Pai L-M, Lee C-K. Fine-tuning of type I interferon response by STAT3. Frontiers in immunology. 2019; 10:1448.
15. Agasing A, Quinn JL, Kumar G, Axtell RC. Interferon-β Intensifies Interleukin-23-Driven Pathogenicity of T Helper Cells in Neuroinflammatory Disease. Cells. 2021;10(8):2139.
16. Agasing AM, Gawde S, Siebert N, Ruprecht K, Paul F, Axtell RC. IFN-β induces IL-6 production by B cells to promote TH17-mediated neuroinflammation. Am Assoc Immnol; 2019.
17. Pons V, Rivest S. Beneficial roles of microglia and growth factors in MS, a brief review. Frontiers in Cellular Neuroscience. 2020;14.
18. Jamshidian A, Abd-Nikfarjam B, Khademi Z, Shaygannejad V, Salehi M. Therapeutic plasma exchange may adjust IL-6 and TGF-β signals in relapsed MS patients peripheral blood. Journal of clinical apheresis. 2020;35(2):72-8.
19. Jamshidian A, Gharagozloo M. Can plasma exchange therapy induce regulatory T lymphocytes in multiple sclerosis patients? Clinical & Experimental Immunology. 2012;168(1):75-7.
20. Jamshidian A, Kazemi M, Shaygannejad V, Salehi M. The effect of plasma exchange on the expression of FOXP3 and RORC2 in relapsed multiple sclerosis patients. Iranian Journal of Immunology. 2015;12(4):311-8.
21. Jamshidian A, Shaygannejad V, Pourazar A, Zarkesh-Esfahani S-H, Gharagozloo M. Biased Treg/Th17 balance away from regulatory toward inflammatory phenotype in relapsed multiple sclerosis and its correlation with severity of symptoms. Journal of neuroimmunology. 2013;262(1-2):106-12.
22. Chen Y-C, Yang X, Miao L, Liu Z-G, Li W, Zhao Z-X, et al. Serum level of interleukin-6 in Chinese patients with multiple sclerosis. Journal of neuroimmunology. 2012;249(1-2):109-11.
23. Patanella AK, Zinno M, Quaranta D, Nociti V, Frisullo G, Gainotti G, et al. Correlations between peripheral blood mononuclear cell production of BDNF, TNF-alpha, IL-6, IL-10 and cognitive performances in multiple sclerosis patients. Journal of Neuroscience Research. 2010;88(5):1106-12.
24. Hemdan NY, Birkenmeier G, Wichmann G, El-Saad AMA, Krieger T, Conrad K, et al. Interleukin-17-producing T helper cells in autoimmunity. Autoimmunity reviews. 2010;9(11):785-92.
25. Shimizu K, Agata K, Takasugi S, Goto S, Narita Y, Asai T, et al. New strategy for MS treatment with autoantigen-modified liposomes and their therapeutic effect. Journal of Controlled Release. 2021.
26. Ramgolam VS, Sha Y, Jin J, Zhang X, Markovic-Plese S. IFN-β inhibits human Th17 cell differentiation. The Journal of Immunology. 2009;183(8):5418-27.
27. Tao Y, Zhang X, Chopra M, Kim M-J, Buch KR, Kong D, et al. The role of endogenous IFN-β in the regulation of Th17 responses in patients with relapsing-remitting multiple sclerosis. The Journal of Immunology. 2014;192(12):5610-7.
28. Prado DS, Cattley RT, Shipman CW, Happe C, Lee M, Boggess WC, et al. Synergistic and additive interactions between receptor signaling networks drive the regulatory T cell versus T helper 17 cell fate choice. Journal of Biological Chemistry. 2021;297(6).