Cytokine 126 (2020) 154911

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Effects of interferon and glatiramer acetate on cytokine patterns in multiple sclerosis patients

Ali Ganji[a,b], Mohsen Ebrahimi Monfared[c], Shima Shapoori[b], Parisa Nourbakhsh[b], Ali Ghazavi[b,d], Keyvan Ghasami[c], Ghasem Mosayebi[a,b,d,*]

[a] Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran
[b] Department of Microbiology and Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
[c] Department of Neurology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
[d] Traditional and Complementary Medicine Research Center (TCMRC), Department of Microbiology and Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran

ARTICLE INFO

Keywords:
Chemokine
Cytokine
Glatiramer acetate
Interferon- β
Multiple Sclerosis

ABSTRACT

Multiple sclerosis (MS) is an unpredictable autoimmune disease, which causes neurodegeneration in the central nervous system. Since the main cause of MS remains obscure, in this study, we aimed to evaluate the serum levels of some cytokines, including interleukin-5 (IL-5), IL-8, IL-9, IL-17A, transforming growth factor-beta (TGF-β), and interferon-gamma (IFN-γ) in relapsing-remitting (RR)-MS patients, treated with IFN-β and glatiramer acetate (GA). Serum samples of RR-MS patients, treated with high-dose IFN-β1a, low-dose IFN-β1a, IFN-β1b, and GA, were assessed by ELISA assay and then compared with the results of treatment-naive patients and healthy controls. The findings showed that the serum levels of IL-8, IL-9, and IFN-γ in treatment-naive patients were significantly higher than the healthy controls, while there was no significant difference in terms of other cytokines between the groups. A significant reduction was observed in the levels of IL-9 and IFN-γ, while there was a significant increase in TGF-β level among patients treated with GA. IFN-β1b resulted in a significant decline in the levels of IL-9 and TGF-β. In addition to these findings, some cytokines were positively correlated in different groups. Overall, the present results support the inflammatory and aggravating effects of IL-8, IL-9, and IFN-γ on MS. Furthermore, based on the results reported in the GA treatment group, we suggest GA as an effective treatment for RR-MS patients.

1. Introduction

Multiple sclerosis (MS) is considered a prototypic inflammatory autoimmune disease, associated with neurodegeneration in the central nervous system (CNS). More than 80% of MS patients experience a relapsing-remitting (RR)-MS course with an initial period of exacerbation, followed by substantial remission [1,2]. Although the exact mechanisms of MS remain unknown, disturbance of optimal balance between cytokines and chemokines of autoreactive $CD4^+$ T helper (Th) cell subsets can play a critical role in the pathogenesis of MS [1,3,4].

Proinflammatory cytokines contribute to the typical features of MS pathology, such as oligodendrocyte cell death, axonal degeneration, and neuronal dysfunction [4]. Production of signature cytokines, such as interferon-gamma (IFN-γ), interleukin-5 (IL-5), IL-17A, and IL-9 by Th1, Th2, Th17, and Th9 cells, respectively and reduction of transforming growth factor-beta (TGF-β), secreted by regulatory T cells (Treg cells), are the key immunopathological features of MS. However, there are controversies regarding the role of cytokines in the pathogenesis of MS [5–10].

There are several disease-modifying drugs with known mechanisms, such as IFN-β and glatiramer acetate (GA), which can be used in the management of MS [11]. Since MS patients are variable in response to therapeutic doses of IFN-β or GA, scholars are motivated to provide more detailed information about the impact of different treatments on the unrecognized or controversial aspects of the immune system [12]. In order to find additional information on immune mediators, levels of cytokines, including IL-5, IL-8, IL-9, IL-17A, TGF-β, and IFN-γ, were assayed in active RR-MS patients treated with GA, IFN-β1a, or IFN-β1b.

* Corresponding author at: Professor of Immunology, Molecular and Medicine Research Center, Arak University of Medical Sciences, P.O. Box: 38481-7-6941, Arak, Iran.
E-mail addresses: a.ganji@arakmu.ac.ir (A. Ganji), ghazaviali@arakmu.ac.ir (A. Ghazavi), k.ghasami@arakmu.ac.ir (K. Ghasami), ghasemmosayebi@arakmu.ac.ir (G. Mosayebi).

https://doi.org/10.1016/j.cyto.2019.154911
Received 11 May 2019; Received in revised form 23 October 2019; Accepted 24 October 2019

Therefore, cytokine profiling presents new approaches for individualized and optimized therapeutic strategies in MS patients.

2. Materials and methods

2.1. Study population

A total of 105 Iranian patients with RR-MS, who had received MS treatments for one to two years, were selected for cytokine profiling in Valiasr Hospital of Arak, Iran. MS was diagnosed by a neurologist according to the McDonald criteria [11]. MS patients with the Expanded Disability Status Scale (EDSS) scores of 0.5–3.5 were diagnosed based on clinical examination, magnetic resonance imaging (MRI), and cerebrospinal fluid (CSF) analysis, including immunoglobulin G (IgG) index and oligoclonal IgG band. Treatment-naive patients received no immunosuppressive or immunomodulatory agents at least three months before sampling. On the other hand, patients with infectious, neoplastic, or autoimmune diseases were excluded from the study. Written informed consent was obtained from all participants, and the experimental protocol for blood collection was approved by the Ethics Committee of Arak University of Medical Sciences (No: IR.ARAKMU.REC.96.93).

2.2. Study design

The selected patients were divided into five groups according to the treatment type (Table 1). The groups included 21 patients treated with low-dose IFN-β1a (CinnoVex, CinnaGen Co., Tehran, Iran), 19 patients treated with high-dose IFN-β1a (ReciGen, CinnaGen Co., Tehran, Iran), 21 patients treated with IFN-β1b (Ziferon, Zistdaru Danesh Co., Tehran, Iran), 17 patients treated with GA (Zahravi Pharmaceutical Co., Tabriz, Iran), and 27 newly diagnosed patients without any treatments (treatment-naive). In addition, 20 age-and sex-matched control subjects were recruited in this study.

2.3. Cytokine detection

On the next day of treatment, blood samples were collected from all patients in tubes without any anticoagulant. Next, they were centrifuged at 3000 × g for 10 min at 4 °C and stored at −80 °C until cytokine determination. Serum levels of IL-5 (sensitivity: 1.5 pg/mL), IL-8 (sensitivity: 2.0 pg/mL), IL-9 (sensitivity: 0.5 pg/mL), IL-17A (sensitivity: 0.5 pg/mL), TGF-β (sensitivity: 8.6 pg/mL), and IFN-γ (sensitivity: 0.99 pg/mL) were determined using an enzyme-linked immunosorbent assay (ELISA; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's guidelines. Each sample was tested in triplicate and quantified using a microplate reader (Stat Fax 2100, USA) at a wavelength of 450 nm.

2.4. Statistical analysis

Data were analyzed in SPSS version 16.0 (SPSS Inc., Chicago, Illinois, USA). Assumption of normality was tested using Kolmogorov–Smirnov test. ANOVA test was used to evaluate differences between variables in each of the studied groups. The mean values were compared between the groups using post hoc Tukey's test. Pearson's Chi-square test was also used to compare the gender and age of different groups. Correlations were evaluated by calculating Pearson's correlation coefficients. Data are presented as mean ± SD. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Serum concentrations of cytokines

Analysis of serum concentrations of IL-5, IL-8, IL-9, IL-17A, TGF-β, and IFN-γ in MS patients and healthy controls is presented in Table 2.

The serum concentration of IL-9 significantly increased in treatment-naive patients, compared to the healthy controls ($P = 0.001$). The level of IL-9 was significantly lower in patients treated with IFN-β1b ($P = 0.041$) and GA ($P = 0.034$), compared to treatment-naive patients (Fig. 1A). Statistical analyses revealed insignificant differences in IL-17A level between the groups (Fig. 1B).

According to Fig. 1C, detectable levels of TGF-β in patients treated with GA were significantly higher than treatment-naive patients ($P = 0.004$). In contrast, TGF-β level in patients treated with IFN-β1b was significantly lower than treatment-naive patients ($P = 0.03$). According to Fig. 1D, there was no significant difference regarding IL-5 concentration between the groups. As indicated in Fig. 1E, serum level of IFN-γ in patients treated with GA was significantly lower than MS patients without treatment ($P = 0.035$). Furthermore, level of this cytokine significantly increased in treatment-naive patients, compared to the healthy controls ($P = 0.028$).

Based on the analysis of serum IL-8 concentration, there was no significant difference between the treatment groups. However, a significant difference was found between the control and treatment-naive groups in terms of serum IL-8 concentration ($P = 0.001$) (Fig. 1F). Furthermore, Figs. 2–6 show positive correlations between cytokine levels in different groups.

4. Discussion

In this study, we investigated the serum concentrations of IL-5, IL-8, IL-9, IL-17A, TGF-β, and IFN-γ in RR-MS patients, who were treated with GA, IFN-β1a, and IFN-β1b. Based on the present results, we found a considerable imbalance between these cytokines in the selected subjects.

Th17 is one of the prominent cell types in the pathogenesis of MS, which induces inflammatory activities by producing IL-17A. In the majority of previous studies, IL-17A level has been reported to increase in treatment-naive MS patients [13]. However, in the present study, there was no significant difference in terms of the level of IL-17A between treatment-naïve and healthy subjects. Contrary to our assumption in this study, a moderate positive correlation was found between IFN-γ, IL-9, and IL-17 cytokines in MS patients treated with GA. No significant difference was found regarding IL-17A level between patients treated with IFN-β and untreated patients, while according to previous studies, IFN-β reduces the level of IL-17A in MS patients [14].

Table 1
Demographic and clinical characteristics of MS patients.

	Healthy control	Treatment-naive	High-dose IFN-β1a	Low-dose IFN-β1a	IFN-β1b	GA	P-value
M/F	5/15	7/20	3/16	2/19	4/17	2/15	NS
Age, years (mean ± SD)	31.2 ± 2.1	30.6 ± 9.5	35.3 ± 11.7	34.8 ± 9.1	32.8 ± 8.5	32.1 ± 6.3	NS
Disease duration, years	–	Newly diagnosed	1–2	1–2	1–2	1–2	NS
EDSS	–	0.5–3.5	0.5–3.5	0.5–3.5	0.5–3.5	0.5–3.5	NS
Relapse[a]	–	1	1	1	1	1	NS

GA: glatiramer acetate; SD: standard deviation; M: male; F: female; NS: not significant; EDSS: expanded disability status scale.
[a] Relapse in the past six months before the study.

Table 2
Serum IL-5, IL-8, IL-9, IL-17A, TGF-β, and IFN-γ concentrations (pg/mL) in MS patients and healthy controls.

	Cytokines					
Groups	IL-9	IL-17A	IFN-g	TGF-β	IL-5	IL-8
Treatment-naive	535.8 ± 139.4	290.0 ± 22.3	346.4 ± 287.7	60.1 ± 48.7	271.7 ± 59.6	615.9 ± 155.1
IFN-β1a (low dose)	560.5 ± 174.0	316.3 ± 21.7	325.7 ± 210.8	59.6 ± 26.2	271.1 ± 36.5	610.7 ± 123.2
IFN-β1a (high dose)	512.7 ± 160.6	347.0 ± 97.0	343.1 ± 211.2	53.9 ± 16.1	287.4 ± 80.4	624.1 ± 128.3
IFN-β1b	448.8 ± 96.6	326.6 ± 51.1	254.6 ± 102.3	44.4 ± 19.6	252.4 ± 48.5	556.7 ± 80.9
GA	432.2 ± 155.9	301.0 ± 21.5	220.4 ± 88.4	68.4 ± 36.8	266.5 ± 46.7	556.8 ± 124.1
Healthy control	371.8 ± 78.4	332.0 ± 22	223.3 ± 85.17	61.6 ± 22.3	268.9 ± 36.3	386.7 ± 132.6

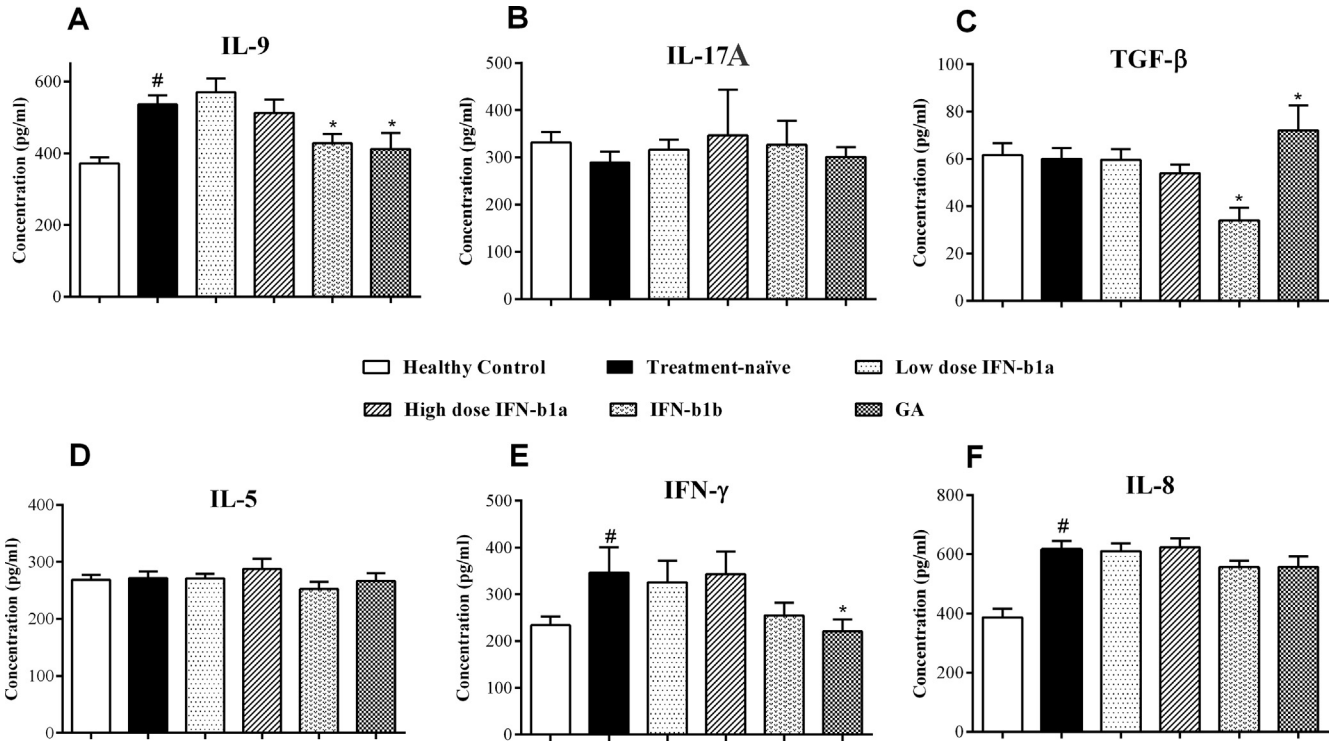

Fig. 1. Detection of IL-9 (A), IL-17A (B), TGF-β (C), IL-5 (D), IFN-γ (E), and IL-8 (F) by ELISA assay in the serum of treatment-naive and RR-MS patients treated with GA and different types of IFN-β (*P < 0.05, significant difference with treatment-naïve patients; #P < 0.05, significant difference with healthy controls).

Th9 cell is another cell, which plays an important role in the pathogenesis of MS by producing IL-9 [15]. The results of IL-9 measurement showed an increase in treatment-naive patients. Previous studies have also confirmed the pathogenic and destructive role of this cytokine in MS [16,17]. However, there are some studies indicating IL-9 reduction in treatment-naive MS patients [14]. This reduction can be attributed to the moderate positive correlation between Th9 and Treg cells through which Treg cells can further reduce the activity of Th9 cells.

Among treatments included in the present study, IFN-β1b and GA could significantly reduce the level of IL-9; however, IFN-β1a had no significant effects on IL-9. The insignificant alteration of IL-9 level may be due to the synergistic effects of Th9 and Th1 cells, as indicated by their positive correlation. IFN-β1b may decrease the pathological response of the immune system through various mechanisms, such as reduction of major histocompatibility complex-II (MHC-II) and B7

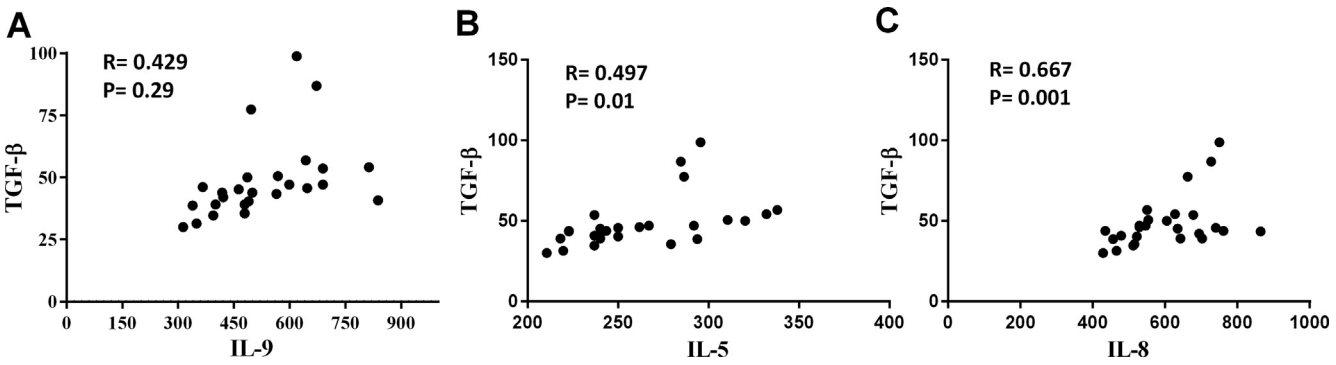

Fig. 2. Significant correlations between (A) TGF-β and IL-9; (B) TGF-β and IL-5; and (C) TGF-β and IL-8 in the treatment-naive group (R = regression; P = P-value).

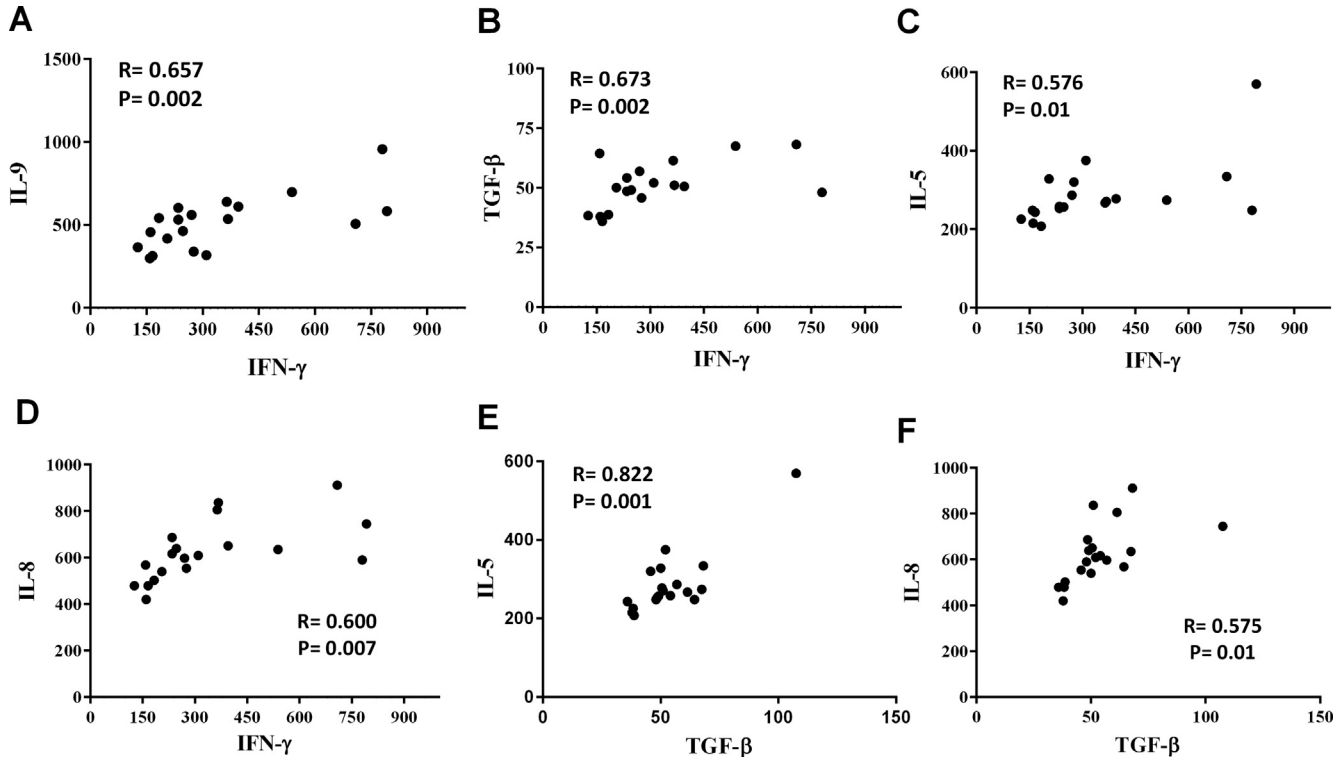

Fig. 3. Significant correlations between (A) IL-9 and IFN-γ; (B) TGF-β and IFN-γ; (C) IL-5 and IFN-γ; (D) IL-8 and IFN-γ; (E) IL-5 and TGF-β; and (F) IL-8 and TGF-β in high-dose IFN-β1a group (R = regression; P = P-value).

expression, induction of T-cell anergy, and reduction of T lymphocyte proliferation [18].

The exact mechanism of the effect of IFN-β1b on IL-9 is not well understood. Although further studies are needed in this context, the most important effect of this drug may be the production of a lower concentration of IL-9 by reducing lymphocyte proliferation. The mechanism of IL-9 reduction by GA can be related to the inactivation of T lymphocytes and prohibition of inflammatory cytokine production by T cells through binding to MHC molecules [4]. On the other hand, according to the observed correlations, a significant increase in TGF-β level could lead to a remarkable reduction in the concentration of IL-9 in the GA-treated group.

Meanwhile, Th17 cells can be one of the sources of IL-9 production in the presence of TGF-β [15]. IL-9, along with IL-21, through autocrine effects on Th17 cells, leads to an increase in differentiation of Th17 cells [19]. Therefore, any intervention that reduces IL-17A and IL-9 levels can help improve MS. In this regard, a previous study showed that in IL-9- or IL-9 receptor-deficient mice, experimental allergic encephalomyelitis (EAE) improved due to the disrupted differentiation of Th17 cells and macrophages [20].

IFN-γ, as one of the most important Th1 cell cytokines with a crucial role in MS pathogenesis, is another cytokine measured in this study [21]. In the present study, IFN-γ concentration significantly increased in treatment-naive patients, which is consistent with previous studies [21]. In addition, GA treatment could significantly reduce IFN-γ, which is an important finding consistent with previous research, indicating the inhibitory effects of GA on IFN-γ producing cells [22].

Th2 cells are another group of T lymphocytes with important roles in the immune system and autoimmune diseases through production of cytokines, such as IL-4 and IL-5. In the present study, IL-5 was evaluated in RR-MS patients. In line with some previous studies, no significant difference was found between the groups [23]. On the other hand, some studies have reported an increase in IL-5 concentration in response to GA therapy [24,25]. In this regard, Sanna et al. revealed that treatment of dendritic cells with GA could lead to an increase in IL-5 production from lymphocytes [26]. The results of this study showed no changes in the level of IL-5 between different treatment groups of RR-MS patients, while other results showed an increase in the serum level of IL-5 in different treatment groups [24–26].

Treg cell is another cell type involved in MS, which acts through

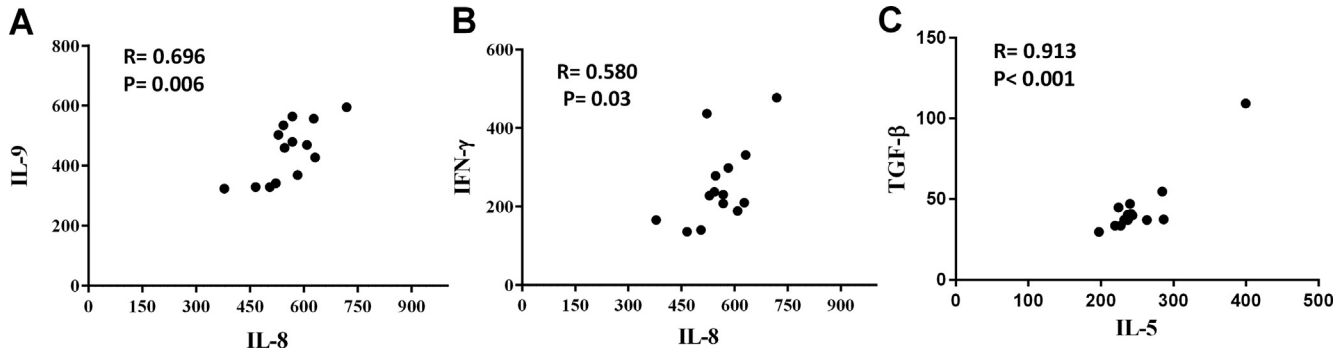

Fig. 4. Significant correlations between (A) IL-9 and IL-8; (B) IFN-γ and IL-8; and (C) TGF-β and IL-5 in the IFN-β1b group (R = regression; P = P-value).

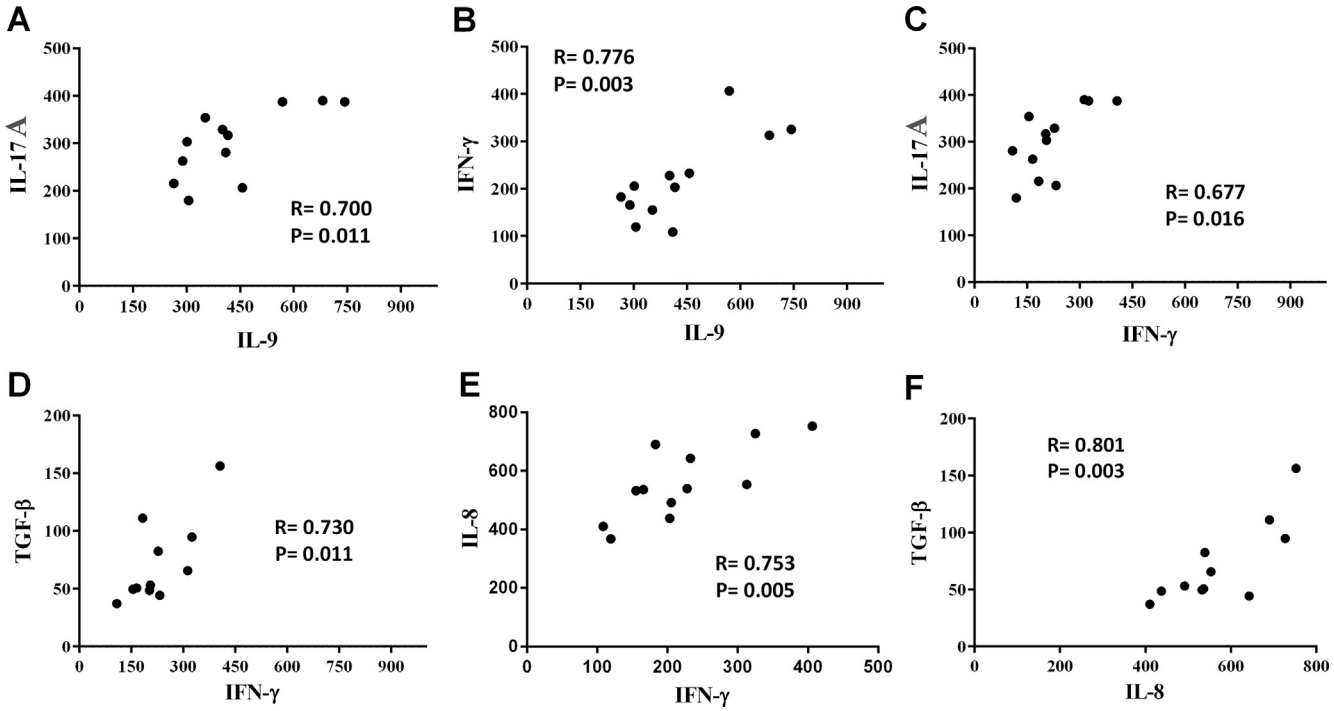

Fig. 5. Significant correlations between (A) IL-17A and IL-9; (B) IFN-γ and IL-9; (C) IL-17A and IFN-γ; (D) TGF-β and IFN-γ; (E) IL-8 and IFN-γ; and (F) TGF-β and IL-8 in the GA group (R = regression; P = P-value).

production of anti-inflammatory cytokines, such as TGF-β and IL-10. Although there was no significant difference in TGF-β level between the control and treatment-naive groups, the serum level of this cytokine was significantly lower in patients treated with IFN-β1b, whereas treatment with GA significantly increased the level of this cytokine. In contrast, other studies showed no changes in TGF-β level among MS patients treated with IFN-β1b [14]. In the present study, there were no significant changes in treatment groups receiving IFN-β1a, while other studies have reported an increase in TGF-β concentration in MS patients treated with IFN-β1a [27]; therefore, further studies are needed to explain this contradiction. In addition, the positive correlation of TGF-β with other measured cytokines in this study indicates the regulatory role of TGF-β in other subsets of T lymphocytes.

IL-8 (CXCL8) is also involved in the pathogenesis of MS via transmigration of lymphocytes across the blood-brain barrier (BBB) [28,29]. In this study, the level of IL-8 in treatment-naive patients was significantly higher than that of healthy subjects. Likewise, other studies have shown higher serum levels of IL-8 in treatment-naive patients,

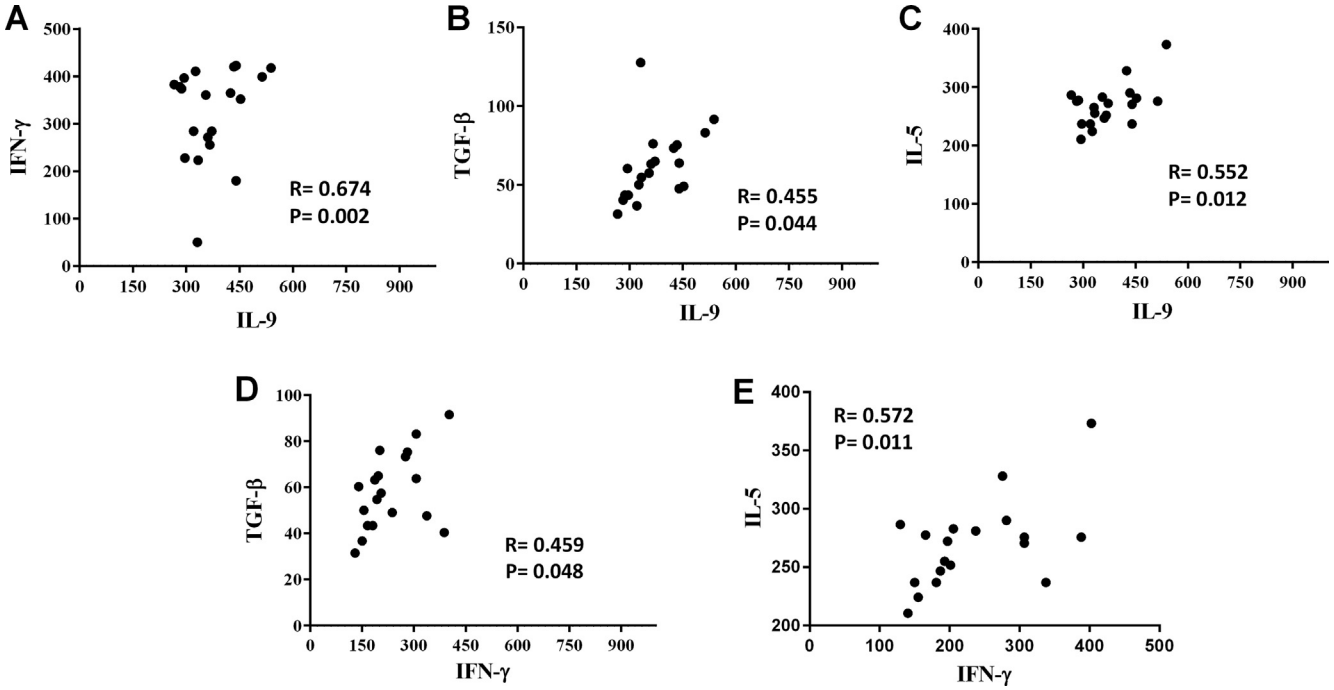

Fig. 6. Significant correlations between (A) IFN-γ and IL-9; (B) TGF-β and IL-9; (C) IL-5 and IL-9; (D) TGF-β and IFN-γ; and (E) IL-5 and IFN-γ in the healthy control group (R = regression; P = P-value).

compared to the control group [29,30]. There were no significant differences between groups treated with IFN-β1 and GA. Contrary to the results of the present study, Brett T. Lund et al. showed that the serum level of IL-8 decreased significantly in MS patients after IFN-β1a therapy [29]. This insignificant alteration in IL-8 concentration between different treatment groups needs to be elucidated in future studies.

In conclusion, the high serum levels of IL-9, IFN-γ, and IL-8 in treatment-naive RR-MS patients confirm the inflammatory and aggravating impact of these cytokines in MS. According to the present results, IFN-β1b and GA therapy led to a significant reduction in the serum level of IL-9, which can be a vital factor in the process of disease management. Besides, GA treatment had significant effects on the reduction of IFN-γ level, while increasing the level of TGF-β. Therefore, it can be considered as an effective treatment for RR-MS patients. However, we observed the negative effect of IFN-β1b on the level of TGF-β, which requires further analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank all the participants of this study, especially the patients for their cooperation. We also extend our gratitude to the Research Council of Arak University of Medical Sciences.

References:

[1] C.A. Dendrou, L. Fugger, M.A. Friese, Immunopathology of multiple sclerosis, Nat. Rev. Immunol. (2015).
[2] W. Elyaman, S.J. Khoury, Th9 cells in the pathogenesis of EAE and multiple sclerosis, Seminars in immunopathology, Springer (2017) 79–87.
[3] M. Sospedra, R. Martin, Immunology of multiple sclerosis, Seminars in neurology, Thieme, Med. Publ. (2016) 115–127.
[4] A. Trenova, G. Slavov, Cytokines in multiple sclerosis–possible targets for immune therapies, J. Neurol Exp. Neurosci. 1 (2) (2016) 25–29.
[5] F. Jadidi-Niaragh, A. Mirshafiey, Th17 cell, the new player of neuroinflammatory process in multiple sclerosis, Scandinavian J. Immunol. 74 (1) (2011) 1–13.
[6] G. Ruocco, S. Rossi, C. Motta, G. Macchiarulo, F. Barbieri, M. De Bardi, G. Borsellino, A. Finardi, M.G. Grasso, S. Ruggieri, T helper 9 cells induced by plasmacytoid dendritic cells regulate interleukin-17 in multiple sclerosis, Clin. Sci. 129 (4) (2015) 291–303.
[7] Y. Sonobe, S. Jin, J. Wang, J. Kawanokuchi, H. Takeuchi, T. Mizuno, A. Suzumura, Chronological changes of CD4+ and CD8+ T cell subsets in the experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis, Tohoku J. Experimen. Med. 213 (4) (2007) 329–339.
[8] J.J. Lafaille, F. Van de Keere, A.L. Hsu, J.L. Baron, W. Haas, C.S. Raine, S. Tonegawa, Myelin basic protein–specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease, J. Experimen. Med. 186 (2) (1997) 307–312.
[9] J.S. Tzartos, M.A. Friese, M.J. Craner, J. Palace, J. Newcombe, M.M. Esiri, L. Fugger, Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis, Am. J. Pathol. 172 (1) (2008) 146–155.
[10] J. Huan, N. Culbertson, L. Spencer, R. Bartholomew, G.G. Burrows, Y.K. Chou, D. Bourdette, S.F. Ziegler, H. Offner, A.A. Vandenbark, Decreased FOXP3 levels in multiple sclerosis patients, J. Neurosci. Res. 81 (1) (2005) 45–52.
[11] M. Ebrahimimonfared, A. Ganji, S. Zahedi, P. Nourbakhsh, K. Ghasami, G. Mosayebi, Characterization of regulatory T-cells in multiple sclerosis patients treated with interferon beta-1a, CNS Neurolog. Dis. Drug Targets 17 (2) (2018) 113–118.
[12] S. Mahurkar, V. Suppiah, C. O'Doherty, Pharmacogenomics of interferon beta and glatiramer acetate response: a review of the literature, Autoimmun. Rev. 13 (2) (2014) 178–186.
[13] A.G. Trenova, G.S. Slavov, M.G. Manova, M.N. Draganaova-Filipova, N.G. Mateva, L.D. Miteva, S.A. Stanilova, Alterations in serum levels of IL-17 in contrast to TNF-alpha correspond to disease-modifying treatment in relapsing-remitting multiple sclerosis, Scandinavian J. Clin. Lab. Investigat. 77 (4) (2017) 283–288.
[14] M. Kürtüncü, E. Tüzün, R. Türkoğlu, B. Petek-Balcı, S. İçöz, M. Pehlivan, Ö. Birişik, C. Ulusoy, E. Shugaiv, G. Akman-Demir, M. Eraksoy, Effect of short-term interferon-β treatment on cytokines in multiple sclerosis: significant modulation of IL-17 and IL-23, Cytokine 59 (2) (2012) 400–402.
[15] G. Beriou, E.M. Bradshaw, E. Lozano, C.M. Costantino, W.D. Hastings, T. Orban, W. Elyaman, S.J. Khoury, V.K. Kuchroo, C. Baecher-Allan, D.A. Hafler, TGF-β Induces IL-9 Production from human Th17 Cells, J. Immunol. 185 (1) (2010) 46–54.
[16] H. Li, B. Nourbakhsh, B. Ciric, G.-X. Zhang, A. Rostami, Neutralization of IL-9 ameliorates experimental autoimmune encephalomyelitis by decreasing the effector T cell population, J. Immunol. 185 (7) (2010) 4095–4100.
[17] R. Yazdani, S. Shapoori, M. Rezaeepoor, R. Sanaei, M. Ganjalikhani-Hakemi, G. Azizi, W. Rae, A. Aghamohammadi, N. Rezaei, Features and roles of T helper 9 cells and interleukin 9 in immunological diseases, Allergolog. Immunopatholog. 47 (1) (2019) 90–104.
[18] B.G. Arnason, Interferon beta in multiple sclerosis, Clin. Immunol. Immunopathol. 81 (1) (1996) 1–11.
[19] E.C. Nowak, R.J. Noelle, Interleukin-9 as a T helper type 17 cytokine, Immunology 131 (2) (2010) 169–173.
[20] E.C. Nowak, C.T. Weaver, H. Turner, S. Begum-Haque, B. Becher, B. Schreiner, A.J. Coyle, L.H. Kasper, R.J. Noelle, IL-9 as a mediator of Th17-driven inflammatory disease, J. Experimen. Med. 206 (8) (2009) 1653–1660.
[21] S. Dhib-Jalbut, M. Chen, K. Henschel, D. Ford, K. Costello, H. Panitch, Effect of combined IFNb-1a and glatiramer acetate therapy on GA-specific T-cell responses in multiple sclerosis, Multiple Sclerosis J. 8 (6) (2002) 485–491.
[22] R.M. Valenzuela, K. Costello, M. Chen, A. Said, K.P. Johnson, S. Dhib-Jalbut, Clinical response to glatiramer acetate correlates with modulation of IFN-gamma and IL-4 expression in multiple sclerosis, Multiple Sclerosis (Houndmills, Basingstoke, England) 13 (6) (2007) 754–762.
[23] J. Sellner, I. Greeve, O. Findling, C.P. Kamm, C. Minten, B. Engelhardt, D. Grandgirard, S.L. Leib, H.P. Mattle, Effect of interferon-β and atorvastatin on Th1/Th2 cytokines in multiple sclerosis, Neurochem. Int. 53 (1–2) (2008) 17–21.
[24] E. Wiesemann, J. Klatt, C. Wenzel, F. Heidenreich, A. Windhagen, Correlation of serum IL-13 and IL-5 levels with clinical response to Glatiramer acetate in patients with multiple sclerosis, Clin. Experimen. Immunol. 133 (3) (2003) 454–460.
[25] E. Wiesemann, J. Klatt, D. Sönmez, R. Blasczyk, F. Heidenreich, A. Windhagen, Glatiramer acetate (GA) induces IL-13/IL-5 secretion in naive T cells, J. Neuroimmunol. 119 (1) (2001) 137–144.
[26] A. Sanna, M.L. Fois, G. Arru, Y.M. Huang, H. Link, M. Pugliatti, G. Rosati, S. Sotgiu, Glatiramer acetate reduces lymphocyte proliferation and enhances IL-5 and IL-13 production through modulation of monocyte-derived dendritic cells in multiple sclerosis, Clin. Experimen. Immunol. 143 (2) (2006) 357–362.
[27] J. Losy, G. Michałowska-Wender, In vivo effect of interferon-β 1a on interleukin-12 and TGF-β1 cytokines in patients with relapsing–remitting multiple sclerosis, Acta Neurolog. Scandinavica 106 (1) (2002) 44–46.
[28] M. Comabella, S.J. Khoury, Immunopathogenesis of multiple sclerosis, Clin. Immunol. 142 (1) (2012) 2–8.
[29] B.T. Lund, N. Ashikian, H.Q. Ta, Y. Chakryan, K. Manoukian, S. Groshen, W. Gilmore, G.S. Cheema, W. Stohl, M.E. Burnett, Increased CXCL8 (IL-8) expression in multiple sclerosis, J. Neuroimmunol. 155 (1–2) (2004) 161–171.
[30] A. Malekzadeh, H. Twaalfhoven, N.J. Wijnstok, J. Killestein, M.A. Blankenstein, C.E. Teunissen, Comparison of multiplex platforms for cytokine assessments and their potential use for biomarker profiling in multiple sclerosis, Cytokine 91 (2017) 145–152.