Neurology Asia 2024; 29(3) : 763 – 771

Investigation of the effects of IL-13 and IL-22 cytokine levels on disease activity, prognosis, and treatment response in multiple sclerosis patients treated with fingolimod and glatiramer acetate

[1]Fidel Demir, [2]Deniz Evrim Kavak, [3]Fırat Karaaslan, [3]Mehmet Ufuk Aluçlu, [4]Sevgi Irtegun Kandemir

[1]Department of Neurology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey; [2]Deniz Evrim Kavak, Department of Medical Biology, Faculty of Medicine, Dokuz Eylul University, İzmir, Turkey; [3]Fırat Karaaslan, Department of Neurology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey; [4]Department of Medical Biology, Faculty of Medicine. Dicle University, Diyarbakır, Turkey

Abstract

Background: Multiple Sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease. In this study, we investigated serum protein levels of Interleukin-13 (IL-13) and Interleukin-22 (IL-22) cytokines. These cytokines play an important role in the generation and regulation of the inflammatory response, which is the pathogenesis of MS, and are potential biomarkers for monitoring therapeutic response. cytokines may play a role in the development of MS lesions. *Methods:* The study included 66 MS patients and 22 healthy individuals. IL-13 and IL-22 cytokine protein levels were measured by ELISA from peripheral blood serum samples collected from the participants. Patient demographics and treatment history data were also collected. *Results:* IL-13 and IL-22 parameters were lower in MS patients compared to the control group. There was a significant difference between the patient and control groups in terms of IL-13 ($p<0.001$). Although the mean IL-22 level of the control group was higher than the patient group, the difference did not reach a significant level (p: 0.257).
Conclusion: The results of the study suggest that IL-13 and IL-22 cytokines play an important role in the pathogenesis of MS and are affected by fingolimod and glatiramer acetate treatment.

Keywords: Multiple sclerosis, interleukin-13, interleukin-22, fingolimod, glatiramer acetate

INTRODUCTION

Multiple Sclerosis (MS) is an inflammatory, demyelinating, and neurodegenerative disease that predominantly affects young adults. Myelin sheaths, oligodendrocytes, and to a lesser extent, axon and nerve cell damage have been reported in MS.[1] It is the most common cause of non-traumatic disability in young adults, affecting more than 2.5 million worldwide.[2] MS, similar to other autoimmune diseases, is more prevalent in women. In its most common form, relapsing-remitting MS (RRMS), the male-to-female ratio has been reported to be approximately 1:2–3. MS typically exhibits clinical features that progress through acute attacks, presenting with symptoms such as optic neuritis, diplopia, sensory impairment, motor weakness, ataxia, and vertigo. Some patients can transition to a progressive form of the disease after 10–20 years of illness.[3]

Although the exact etiology of the disease remains uncertain, there is substantial evidence suggesting that the immune system plays a crucial role in its pathogenesis. Cytokines are pivotal in regulating the immune system and numerous immune signaling centers are involved in maintaining the balance between inflammatory and anti-inflammatory cytokines.[4] Several cytokines play a role in the pathogenesis of MS. IL-13 belongs to the major anti-inflammatory cytokine family and is effective in inhibiting the production of pro-inflammatory cytokines or counteracting the biological effects of pro-inflammatory mediators.[5,6] In an experimental study, IL-13 has been shown to play a neuroprotective role in MS.[7] IL-22 has been associated with chronic inflammation. Interestingly, despite its

Address correspondence to: Fidel Demir, Department of Neurology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey. E-mail: fideldemir2605@gmail.com
Date of Submission: 16 April 2024; Date of Acceptance: 4 May 2024
https://doi.org/10.54029/2024pcz

inflammatory role in autoimmune diseases, IL-22 also exhibits a protective function in preventing inflammation in other diseases.[8,9] IL-22 disrupts the integrity of the blood brain barrier, enabling lymphocyte entry into the CNS, raising the possibility that this cytokine may contribute to MS severity.[10] In an experimental study on MS, it was shown that IL-22 inhibitor receptor protein exacerbated the disease course. Based on this, it has been suggested that IL-22 cytokine may have an anti-inflammatory function in MS.[11]

Many immunomodulators and immunosuppressive drugs are used in the treatment of MS. Currently, glatiramer acetate (GA) is used as an immunomodulatory drug in first-line therapy and is effective for both innate and acquired immunity.[12] GA clinically reduced the frequency of attacks by 29–32% in patients with RRMS, and radiological effects have been shown to reduce new lesion development and lesion burden.[13] Fingolimod (FTY720) is an immunosuppressive drug used in second-line therapy. Lymphocytes activated by the sphingosine-1 phosphate signal in the immune system are prevented from leaving the lymph node.[14] Besides its anti-inflammatory effects, the neuroprotective effects of fingolimod are well documented.[15] With the use of fingolimod, there was no progression in disability in almost 90% of patients, and the 2-year recurrence rate decreased by more than 90% compared to baseline.[16]

The role of IL-13 and IL-22 cytokines in MS pathogenesis and their potential to be used as biomarkers to test prognosis and treatment efficacy in patients receiving FTY720 or GA are unknown. The main aim of this study is to reveal the possible relationship.

METHODS

Study design and participant selection

Our research included 66 patients who were diagnosed with RRMS, according to the 2017 Revised McDonald criteria, who applied to the Neurology Department at Dicle University Medical Faculty Hospital between March 29, 2020 and June 25, 2020. MS patients are divided into three equal groups according to treatment status. Group F consisted of patients using FTY720 for at least 6 months, while group G consisted of patients using GA for at least 6 months. Patients who did not use any preventive drug therapy constituted the untreated patients (UP) group. Groups F, G and UP consisted of 22 patients. Twenty two healthy individuals without any chronic disease were recruited for the control group. The control group was selected from hospital health workers and students who met the inclusion criteria. Those with another chronic disease, those who received exacerbation treatment within the last month, those outside the age range of 18–55, and other forms of progressive MS were excluded from the study. All patients underwent a detailed neurological examination 30 minutes before blood collection, and the EDSS score was calculated. Gender, age at onset of the first attack, duration of disease, duration of drug use, total number of attacks in the last year, oligoclonal band (OCB) positivity, and IgG index of all patients were recorded.

Cranial and spinal MRIs taken in the last year in MS patients included in the study were examined, and it was determined whether there were lesions in the periventricular, cortical/juxtacortical, infratentorial, and medulla spinalis regions, which are 4 regions specified in McDonald's 2017 spatial extension criteria, and the total number of involved regions was recorded. The study was approved by the local ethics committee and written informed consent was obtained from all participants.

Assessment of therapeutic efficacy

For RRMS patients on modifying medication, the treatment was considered therapeutically effective if there were no attacks within the last 6 months, if the baseline EDSS score was 0 and there was an increase of <1.5 points, or if the baseline EDSS score was ≥1 and there was an increase of <1 point, or if the baseline EDSS score was >5 and there was an increase of <0.5 points at 6 months.[17]

Materials and study method

All experiments within the scope of the study were performed at the Dicle University Science and Technology Application and Research Center (DUBTAM).

Serum isolation from peripheral blood and measurement of IL-13 and IL-22 by ELISA method

Three ml of venous blood obtained from MS patients and healthy individuals was taken into serum tubes. After waiting for 30 minutes, the blood samples were centrifuged at 2000 x g for 15 minutes (Thermo Scientific SL8, Waltham, USA), and the serum samples were stored at -80 °C until an ELISA was performed. IL-13 (Sunredbio kit) and IL-22 (Elabscience® kit) cytokine levels in serum were measured using ELISA kits. ELISA experiments were performed according to the manufacturer›s instructions. The sensitivity for

Table 1: Gender, Mean age, age at the first attack, duration of disease, and EDSS scores of study groups

	Gender (F/M)	Age (year), (Mean±SD)	BMI, (Mean±SD)	Age at First Attack (Year), (Mean±SD)	Duration of Disease (month) (Mean±SD)	EDSS Score, (Mean±SD)
UP Group	15/7	29.34±7.82	23.4±3.5	26.17±5.86	32.24±39.54	1.54±0.56
F Group	16/6	33.6±8.70	24.3±3.0	27.08±6.94	81.04±34.06	2.62±1.24
G Group	14/8	32.6±9.16	23.7±3.6	27.92±8.21	55.36±35.17	1.96±1.18
Control Group	15/7	32.28±8.16	24.0±4.5	---	---	---
Total	**60/28**	**31.75**	**23.9±3.6**	**26.77**	**56.05**	**2.04**

G: Glatiramer acetate, F: Fingolimod, MS: MS patients without treatment, EDSS: Expanded Disability Status Scale, SD: Standart Deviation, BMI: Body Mass Index

IL-13 is 0.413 pg/mL, while the sensitivity for IL-22 is 9.38 pg/mL. The absorbance was measured at 450 nm with a plate reader spectrophotometer (Multi Scan Go, Thermo).

Statistical analysis

Version 25.0 of the SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) software was used for statistical analysis of the data. Either Chi-squared test or Mann–Whitney U test was used to compare measures between two groups, where appropriate. One-way ANOVA or Kruskal–Wallis test was used for comparisons between three or more groups, where appropriate. Pearson and Spearman correlations were used and the correlation coefficient was indicated as 'r'. The results were evaluated within the 95% confidence interval and $p<0.05$ was defined as statistically significant.

RESULTS

A total of 88 participants (60 women and 28 men) were included in the study and the patients with MS group consisted of 66 people (45 women and 21 men). The mean age of the patient group was 31.53 years and the mean disease duration was 56.05 months. The mean age at onset of the first attack was 26.77 years, with a minimum of 14 and a maximum of 47 years. The main characteristics of the study groups shown in Table 1.

It was determined that there was a significant difference in terms of IL-13 levels between the patient and control groups ($p<0.001$). The mean IL-13 levels in the control group (52.5±28.33 pg/mL) were significantly higher than the levels in the patients (29.22±26.27 pg/mL). Although the mean IL-22 levels of the control group (13.73±20.08 pg/mL) were higher than those of the patient group (3.06±3.05 pg/m), the difference was not significant (p: 0.257) (Table 2).

When the groups were compared within themselves, no significant difference was found between the MS patients for IL-13 levels compared to the treatment groups. While IL-22 levels were determined to 'too low to be detected' (close to zero or zero) in 71 (80.7%) of 88 individuals, IL-22 levels could be detected as above zero in only 17 individuals. Of these 17 individuals, 3 were from group G, 4 were from group F, 7 were from the MS group, and 3 were from the control group. When the individuals were compared according to their groups, IL-22 levels were highest in the control group (mean 13.73±20.08 pg/mL), followed by the G group (3.80±4.75 pg/m) and the MS group (3.09±3.08 pg/mL). Group F (mean 2.24±1.69 pg/mL) had the lowest IL-22 level. There was no significant difference between the groups in terms of mean IL-22 levels ($p = 0.714$) (Table 3).

In the evaluation made according to the gender of the participants, while the mean IL-22 level

Table 2: Comparison of the parameters examined in the MS patients and control group examined in the study according to the patient and control groups

	Patient Group (n: 66), Mean ± SD	Control Group (n: 22), Mean ± SD	P value
IL-13, pg/mL	29.22 ± 26.27	52.5 ± 28.33	**0.001**
IL-22, pg/mL	3.06 ± 3.05	13.73 ± 20.08	0.257

Table 3: Distribution of IL-13 and IL-22 cytokine levels by groups

	G Group (n: 22)	F Group (n: 22)	UP Group (n: 22)	Control Group (n: 22)	p value
IL-13	33.25 pg/mL	20.79 pg/mL	33.62 pg/mL	52.5 pg/mL	**0.001**
IL-22	3.80±4.75 pg/mL	2.24±1.69 pg/mL	3.09±3.08 pg/mL	13.73±20.08 pg/mL	0.714

of 7 male individuals in the control group was 6.58±16.33, the mean IL-22 levels in men with MS (n = 21) were found to be 0.23±0.75 pg/mL. When the two groups were compared, although IL-22 production was significantly higher in men in the control group, the difference could not reach a significant level (p: 0.321). In the evaluation for women, the mean IL-22 level was found to be 3.48±3.32 pg/mL in women with MS (n = 45), while this value was determined to 4.44±4.74 in controls. When the two groups were compared, although IL-22 levels were found to be higher in women in the control group, the difference was not significant (p = 0.830). Among the participants, 18 were smokers and 70 were non-smokers. IL-13 levels were similar in the two groups (p: 0.123).

When the treatment effectiveness was evaluated, it was determined that the treatment was effective in 19 of the 22 patients in the G group, while the treatment effectiveness was low in 3 patients. There was no significant difference in IL-13 levels between patients with effective treatment and those with low treatment effectiveness (p = 0.094). While treatment was found to be effective in 17 of 22 patients in group F, treatment effectiveness was found to be low in 5 patients. There was no significant difference in terms of IL-13 levels between patients who were treated effectively and those who were not (p = 0.493). Since the efficacy of treatment was good in all the patients in the G group (n:3) and F group (n:4) with IL-22 levels, no statistical comparison was made between the treatment efficacy and IL-22 levels.

When the first attack complaints of the patients were analysed, mean IL-13 and IL-22 values were similar in 26 patients with paresthesia, 20 patients with ON, 8 patients with vertigo, 8 patients with diplopia and 4 patients with paresis (p values were 0.074 and 0.601, respectively). When the history of attacks in the last year was analysed, 38 patients had no attacks, 24 patients had only one attack and 4 patients had two attacks. When the groups were compared according to the number of attacks, IL-13 and IL-22 cytokine levels were found to be similar (p value for IL-13: 0.428, p value for IL-22: 0.250).

In this study, a significant, negative and moderately strong correlation was found between IL-13 levels and baseline EDSS score (r = -0.421, p = 0.004). There was a significant, negative but weak correlation between IL-13 levels and current EDSS (r = -0.243, p = 0.049). There is no significant relationship between IL-22 levels, the initial EDSS score before the drug, and the current EDSS score (p = 0.115, p = 0.718).

An OCB test was performed in 48 of the 66 RRMS patients included in our study, and IgG index data for only 26 of them were obtained. OCB was positive in 47 of 48 patients. 18 of 26 patients had high IgG index (> 0.7), while 8 patients had low IgG index (< 0.7). IL-13 levels were found to be similar in OCB-positive and OCB-negative patients (p = 0.649). OCB was positive (100%) in all 11 MS patients whose IL-22 levels could be measured. In this respect, statistical comparisons could not be made. IL-13 (p = 0.682) and IL-22 (p = 1.000) levels were found to be similar between patients with high (> 0.7) and low (< 0.7) IgG indexes.

When cranial and spinal MRI scans of the patients were analysed Involvement was found in two sites in 45 (75%), in three sites in 17, in one site in 6 and in four sites in 3 (Table 4). While the number of areas involved in MRI increased from 1 to 4, IL-13 and IL-22 levels were similar (p values were 0.963 for IL-13 and 0.126 for IL-22, respectively). There was no significant relationship between the number of areas involved in MRI and the parameters studied.

VEP Test The relationship between the presence of elongation in P100 latency (P100 latency >115 msec) and operating parameters was also investigated. 62 patients had a VEP test report. According to this, IL-13 and IL-22 cytokine levels and VEP Test P100 latency were found to be similar for patients with elongation in the right eye, elongation in the left eye, elongation in both eyes, and normal in both eyes (p value 0.697 for IL-13, 0.246 for IL-22).

DISCUSSION

The world population is increasing rapidly, and the number of people affected by autoimmune

Table 4: Involvement regions detected in brain MRI according to treatment groups of MS patients

	G Group (n: 22)		F Group (n: 22)		UP Group (n: 22)	
	Number (n)	**Percent (%)**	**Number (n)**	**Percent (%)**	**Number (n)**	**Percent (%)**
One Region	1	4.55%	0	0.00%	5	22.70%
Two Regions	17	77.30%	13	59.10%	11	50.00%
Three Regions	4	18.15%	8	36.35%	4	18.20%
Four Regions	0	0.00%	1	4.55%	2	9.10%
Total	**22**	**100.00%**	**22**	**100.00%**	**22**	**100.00%**

G: Glatiramer acetate, F: Fingolimod, UP: Untreated patients

diseases, such as MS is increasing daily.[18] The mean age of MS onset is between 23.5 and 30 years.[19] In our study, the age of onset of MS was at least 14 and at most 47 years. The mean age of MS onset, that is, the first attack, was determined as 26.77 years, which is consistent with the age range reported in the literature.

The incidence of MS in women is 1.5–3 times higher than in men.[20] Furthermore, the female to male ratio among RRMS patients is 3:1 (21). Of the 66 patients in our study, 42 were women and 24 were men, and these ratios are consistent with the literature. OCB positivity is observed in more than 90% of clinically definite MS cases.[22] In this study, 47 of 48 patients were positive for OCB, which is also consistent with the literature.

MS is a chronic neurodegenerative disease involving autoimmune responses against myelin antigens associated with the activity of proinflammatory cytokines that exert neurotoxic effects through various mechanisms.[23] Neurodegenerative brain damage in MS results from an imbalance between proinflammatory cytokines released by T helper 1 (Th1) and T helper 17 (Th17) cells and anti-inflammatory cytokines released by T helper 2 (Th2) cells.[24] IL-13, secreted mainly by Th2 lymphocytes, is one of the pleiotropic cytokines that can exert immediate inflammatory effects.[25] In the autoimmune encephalomyelitis (EAE) study, an animal model of MS, mice are injected with IL-13. It was then shown that the infiltration of mononuclear cells into the spinal cord of EAE mice was significantly reduced and remyelination was increased.[26] Similarly, in the experimental study conducted by Park *et al*. it was observed that symptoms improved with IL-13 injection in mice with EAE and it was stated that it has therapeutic potential.[27] In another experimental EAE study, following IL-13 immune gene therapy in mice, it was determined by gene expression analysis that IL-13 redirects the polarisation of both brain-resident microglia and infiltrating macrophages to an alternatively activated phenotype, thereby promoting the transformation of a pro-inflammatory environment into an anti-inflammatory environment. IL-13 immune gene therapy can also limit lesion severity in a pre-existing inflammatory environment.[28] IL-13 has been shown to play a neuroprotective role on the cerebral cortex in a study of cerebrospinal fluid (CSF) from MS patients.[29] In the light of the data obtained from these studies, it appears that IL-13 has the potential to improve the disease outcome for MS.

In a study involving 34 untreated RRMS patients, the percentages of IL-13-producing T cells were calculated in cerebrospinal fluid (CSF) samples from patients and healthy subjects. It was determined that the percentage of IL-13-producing T cells was higher in relapsing patients than in patients in remission. A positive correlation was found between IL-13 producing T cells and EDSS.[30] In our study, on the contrary, a significant, negative but weak correlation was found between IL-13 levels and EDSS (r=-0.243, p:0.049). A decrease in IL-13 serum protein levels was observed as EDSS increased.

In a study consisting of 68 Iranian MS patients, IL-13 cytokine levels were found to be associated with MS, and it was stated that low production of IL-13 may predispose to the development of MS.[31] In a study involving 44 Iranian RRMS patients, no significant difference was observed between the patient and control groups in terms of IL-13 serum protein levels. However, IL-13 levels were found to be significantly higher in males in the control group compared to the patient males. The lower IL-13 level in male patients may explain the poorer prognosis in males.[32] In our study, no significant difference was observed in male patients compared to male controls in terms of IL-13 levels. Similarly, no significant difference was observed among women.

Wiesemann *et al*. studied the correlation of serum IL-13 with clinical response in MS patients

given GA. They reported that serum IL-13 levels increased significantly when GA was given to MS patients, whereas there was no increase in serum IL-13 levels in the serum of MS patients who did not receive treatment or MS patients who did not respond to treatment.[33] Likewise, In a study by Sanna *et al*. consisting of 11 RRMS patients, IL-13 level was found to be lower in the patient group compared to the healthy group. IL-13 level was found to be increased in a patient using GA.[34] In our study, the IL-13 cytokine level was found to be significantly higher in the control group, while no difference was observed in the IL-13 cytokine level in MS patients using GA compared to the MS group that did not use drugs.

In an experimental study, clinical and histopathological improvement was observed with the administration of FTY720 to mice with colitis with oxazolone, and this therapeutic efficacy was associated with a significant decrease in serum IL-13 level.[35] In another experimental study, after the treatment of mice with allergic rhinitis with topical intranasal FTY720, a decrease in the level of IL-13 produced from lymph nodes was observed.[36] In our study, the IL-13 level was found to be at its lowest level in MS patients using FTY720 compared to other MS patients. Our study results show that the level of IL-13, an anti-inflammatory cytokine, is decreased in MS patients, and the use of FTY720 also reduces the level of IL-13.

Current data on IL-22 expression in the pathogenesis of MS are quite limited. In a study of 34 RRMS patients, an increase in IL-22 protein levels was found in patient serum during the attack period and it was suggested that IL-22 may play a role in MS attacks.[37] In an experimental EAE study conducted by Lindahl H *et al*. it was determined that IL-22 inhibitory protein played a pathogenic role. From this point of view, IL-22 has been suggested to have a therapeutic potential in MS.[38] In contrast, an experimental study by Kreymborg *et al*. showed that IL-22 is not necessary for the development of EAE.[39] As can be understood from these studies, there are different results between the study data regarding the role of IL-22 in MS.

In a study involving 34 untreated RRMS patients, the percentages of IL-22 producing T cells were calculated in CSF samples obtained from the patient and healthy groups. In this study, the percentage of IL-22 producing T cells was found to be significantly higher in the patient group.[30] In a study involving 141 MS patients, IL-22 serum levels were found to be significantly higher in the patient group than in the control group. In addition, 26 patients with relapsing exacerbations in the last 2 months were accepted as having active MS, and a significant increase in IL-22 level was observed in these patients compared to other non-active MS patients. In the brain biopsy samples of the patients, it was observed that IL-22 targeted astrocytes and increased the survival of the cells. Based on this, it has been suggested that IL-22 may play a neuroprotective role in the pathogenesis of MS.[40] In another study of 22 RRMS patients, it was found that the number of active MS lesions in the MRI brain of the patients was clearly associated with IL-22 production. Thus, it was claimed that serum IL-22 protein levels are associated with lesion burden and poor prognosis in MS.[41] In addition, the increase in IL-22 level in the induction phase and during the period of highest disability in the EAE model and the decrease in IL-22 level in the recovery phase indicate that IL-22 is associated with the course of the disease.[42]

n our study, contrary to the literature, when the control group was taken alone and the other 3 groups were evaluated as the patient group, the mean IL-22 level of the control group (13.73, 20.08) was found to be higher than that of the patient group (3.06, 3.05), but the difference was not significant ($p = 0.257$). In the literature review, no studies related to IL-22 were observed in MS patients using GA. In our study, no significant difference was observed between IL-22 levels (3.80, 4.75 pg/m) in MS patients using GA and MS patients who did not use drugs (3.09, 3.08 pg/mL). In addition, no significant difference was found between women in the control group and female MS patients in terms of IL-22 levels (p: 0.830). Similarly, no significant difference was found between men in the control group and male MS patients (p: 0.321).

In a study involving 16 RRMS patients, peripheral blood mononuclear cells (PBMCs) were isolated from patients before and three months after starting fingolimod treatment. As a result of the analysis, it was determined that IL-22 mRNA expression decreased with fingolimod treatment.[43] In a study by Kürtüncü *et al*. 66 RRMS patients were treated with fingolimod for 6 months and serum samples were collected from the patients at baseline, 3rd and 6th months. When serum IL-22 protein levels were analysed, it was determined that serum IL-22 levels did not change with FTY720.[44] In another study, Serum IL-22 protein levels are examined at 6 and 12 months in RRMS patients receiving FTY720. It was determined that

IL-22 levels decreased significantly with FTY720 ($P < .001$). In addition, a positive correlation was found between IL-22 protein levels and EDSS score. Based on this, it was suggested that IL-22 may be a good marker for the severity of MS disease and the effectiveness of treatment.[45] In our study, although serum IL-22 levels were higher in the non-medicated patient group than in the FTY720-treated patients, the difference was not significant. No significant correlation was found between IL-22 levels and EDSS score ($p = 0.718$).

Smoking is a risk factor for MS; smoking has also been associated with disease activity and overall prognosis in patients with MS. It is reported that disease activity is higher, brain atrophy is faster and the burden of disability is higher in MS patients who smoke.[46] In a study by Correale *et al*. an increase in the number of cells producing IL-13 and IL-22 was observed in MS patients who smoked.[47] In this study, no significant difference was observed between smokers and non-smokers in terms of IL-13 and IL-22 serum protein levels.

The main limitations of this study are that it is a single-center study, it had a small number of participants, and that there was limited financial support.

In conclusion, to the best of our knowledge, this is the first study to date to compare FYT720, GA, and drug-free MS patients with a control group, and to compare IL-13 and IL-22 cytokines between these groups. Our study strongly supports that IL-13 and IL-22 levels are associated with progression in MS patients and that they can be used as an indicator of therapeutic efficacy in patients using medication. In conclusion, our study shows that IL-13 and IL-22 play an important role in the pathogenesis of MS. More comprehensive, multi-center studies should be conducted in the future aiming to eliminate the limitations reported in our study and previous literature studies.

DISCLOSURE

Ethics: This study was approved by the Dicle University Clinical Research Ethics Committee (Date: 25.03.2021, No: 218). Written informed consent was obtained from all study participants.

Financial Support: This study was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) with project number 121S391.

Conflict of interest: None

REFERENCES

1. Goodin D. The epidemiology of multiple sclerosis: insights to a causal cascade. *Handb Clin Neurol* 2016;138:173-206. doi: 10.1016/B978-0-12-802973-2.00011-2.
2. Maier S, Bajkó Z, Roşescu R, *et al.* Sociodemographic and clinical determinants of fatigue in multiple sclerosis. *Life* (Basel) 2023;13 (11):2132. doi: 10.3390/life13112132.
3. Lublin FD, Reingold SC, Cohen JA, *et al*. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology* 2014;83:278-86. doi: 10.1212/WNL.0000000000000560.
4. Shobeiri P, Seyedmirzaei H, Karimi N, *et al*. IL-6 and TNF-α responses to acute and regular exercise in adult individuals with multiple sclerosis (MS): a systematic review and meta-analysis. *Eur J Med Res* 2022;27 (1):185. doi: 10.1186/s40001-022-00814-9.
5. Hekim N, Alkan ŞŞ. In Immunology. Pf: 205. Nobel Medical Bookstore, Istanbul, 2017.
6. Darkhal P, Gao M, Ma Y, Liu D. Blocking high-fat diet-induced obesity, insulin resistance and fatty liver by overexpression of Il-13 gene in mice. *Int J Obes* (Lond) 2015;39 (8):1292-9. doi: 10.1038/ijo.2015.52.
7. Cash E, Minty A, Ferrara P, Caput D, Fradelizi D, Rott O. Macrophage-inactivating IL-13 suppresses experimental autoimmune encephalomyelitis in rats. *J Immunol* 1994;153 (9):4258-67.
8. Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Flavell RA. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity* 2007; 27 (4):647-59. doi: 10.1016/j.immuni.2007.07.023.
9. Radaeva S, Sun R, Pan HN, Hong F, Gao B. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. *Hepatology* 2004; 39 (5):1332-42. doi: 10.1002/hep.20184.
10. Kebir H, Kreymborg K, Ifergan I, *et al*. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* 2007;13 (10):1173-5. doi: 10.1038/nm1651.
11. Laaksonen H, Guerreiro-Cacais AO, Adzemovic MZ, *et al*. The multiple sclerosis risk gene IL22RA2 contributes to a more severe murine autoimmune neuroinflammation. *Genes Immun* 2014;15 (7):457-65. doi: 10.1038/gene.2014.36.
12. Efendi H. Multiple sclerosis diagnosis and treatment guideline. İstanbul: Galenos publishing, 2018: 61-62.
13. Khan O, Rieckmann P, Boyko A, *et al*. Efficacy and safety of a three-times-weekly dosing regimen of glatiramer acetate in relapsing-remitting multiple sclerosis patients: 3-year results of the Glatiramer Acetate Low-Frequency Administration open-label extension study. *Mult Scler* 2017;23 (6):818-29. doi: 10.1177/1352458516664033.
14. Brinkmann V. FTY720 (fingolimod) in multiple sclerosis: therapeutic effects in the immune and the central nervous system. *Br J Pharmacol* 2009;158:1173-82. doi: 10.1111/j.1476-5381.2009.00451.x.
15. Terzi M, Helvacı EM, Şen S, *et al*. Clinical and demographic characteristics and two-year efficacy

and safety data of 508 multiple sclerosis patients with fingolimod treatment. *Noro Psikiyatr Ars* 2023;60 (1):23-27. doi: 10.29399/npa.28081. eCollection 2023.
16. Mitsikostas DD, Orologas A, Dardiotis E, *et al*. A Prospective, observational study assessing effectiveness, safety, and QoL of Greek patients with multiple sclerosis under treatment with fingolimod. *Adv Ther* 2023;40 (5):2217-33. doi: 10.1007/s12325-022-02388-8.
17. Biernacki T, Sandi D, Füvesi J, *et al*. The safety and efficacy of fingolimod: Real-world data from a long-term, non-interventional study on the treatment of RRMS patients spanning up to 5 years from Hungary. *PLoS One* 2022;17 (4):e0267346. doi: 10.1371/journal.pone.0267346.
18. Walton C, King R, Rechtman L, *et al*. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. *Mult Scler* 2020;26 (14):1816-21. doi: 10.1177/1352458520970841.
19. Compston AC, Cole AA. Multiple sclerosis. *Lancet* 2002;359:1221-31. doi: 10.1016/S0140-6736(02)08220-X.
20. Dobson R, Giovannoni G. Multiple sclerosis–a review. *Eur J Neurol* 2019;26:27-40. doi: 10.1111/ene.13819.
21. Kalincik T, Vivek V, Jokubaitis V, *et al.* Sex as a determinant of relapse incidence and progressive course of multiple sclerosis. *Brain* 2013;136:3609-17. doi: 10.1093/brain/awt281.
22. Thompson EJ, Freedman MS. Cerebrospinal fluid analysis in the diagnosis of multiple sclerosis. *Adv Neurol* 2006;98:147-60.
23. Centonze D, Muzio L, Rossi S, *et al*. Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *J Neurosci* 2009;29(11):3442-52. doi: 10.1523/JNEUROSCI.5804-08.2009.
24. Zeis T, Graumann U, Reynolds R, Schaeren-Wiemers N. Normal-appearing white matter in multiple sclerosis is in a subtle balance between inflammation and neuroprotection. *Brain* 2008;131(Pt 1):288-303. doi: 10.1093/brain/awm291.
25. Barten LJ, Allington DR, Procacci KA, Rivey MP. New approaches in the management of multiple sclerosis. *Drug Des Devel Ther* 2010;4:343-66. doi: 10.2147/DDDT.S9331.
26. Azimzadeh M, Mahmoodi M, Kazemi M, *et al*. The immunoregulatory and neuroprotective effects of human adipose derived stem cells overexpressing IL-11 and IL-13 in the experimental autoimmune encephalomyelitis mice. *Int Immunopharmacol* 2020;87:106808.doi: 10.1016/j.intimp.2020.106808.
27. Park J, Choi SW, Cha BG, Kim J, Kang SJ. Alternative activation of macrophages through interleukin-13-loaded extra-large-pore mesoporous silica nanoparticles suppresses experimental autoimmune encephalomyelitis. *ACS Biomater Sci Eng* 2021;7 (9):4446-53. doi: 10.1021/acsbiomaterials.1c00946.
28. Guglielmetti C, Le Blon D, Santermans E, *et al*. Interleukin-13 immune gene therapy prevents CNS inflammation and demyelination via alternative activation of microglia and macrophages. *Glia* 2016;64(12):2181-200. doi: 10.1002/glia.23053.
29. Rossi S, Mancino R, Bergami A, *et al*. Potential role of IL-13 in neuroprotection and cortical excitability regulation in multiple sclerosis. *Mult Scler* 2011;17 (11):1301-12. doi: 10.1177/1352458511410342.
30. Ghezzi L, Cantoni C, Cignarella F, *et al*. T cells producing GM-CSF and IL-13 are enriched in the cerebrospinal fluid of relapsing MS patients. *Mult Scler* 2020;26(10):1172-86. doi: 10.1177/1352458519852092.
31. Seyfizadeh N, Kazemi T, Farhoudi M, *et al*. Association of IL-13 single nucleotide polymorphisms in Iranian patients to multiple sclerosis. *Am J Clin Exp Immunol* 2014;3(3):124-9.
32. Peiravian F, Rajaian H, Samiei A, *et al*. Altered serum cytokine profiles in relapse phase of relapsing-remitting multiple sclerosis. *Iran J Immunol* 2016;13(3):186-96.
33. Wiesemann E, Klatt J, Wenzel C, Heidenreich F, Windhagen A. Correlation of serum IL-13 and IL-5 levels with clinical response to glatiramer acetate in patients with multiple sclerosis. *Clin Exp Immunol* 2003;133(3):454-60. doi: 10.1046/j.1365-2249.2003.02238.x.
34. Sanna A, Fois ML, Arru G, *et al*. Glatiramer acetate reduces lymphocyte proliferation and enhances IL-5 and IL-13 production through modulation of monocyte-derived dendritic cells in multiple sclerosis. *Clin Exp Immunol* 2006;143(2):357-62. doi: 10.1111/j.1365-2249.2006.02997.x.
35. Daniel C, Sartory NA, Zahn N, Schmidt R. FTY720 ameliorates oxazolone colitis in mice by directly affecting T helper type 2 functions. *Mol. Immunol* 2007; 44(13):3305-16. doi: 10.1016/j.molimm.2007.02.026.
36. Kleinjan A, van Nimwegen M, Leman K, Hoogsteden HC, Lambrecht BN. Topical treatment targeting sphingosine-1-phosphate and sphingosine lyase abrogates experimental allergic rhinitis in a murine model. *Allergy* 2013;68 (2):204-12. doi: 10.1111/all.12082.
37. Khaiboullina SF, Gumerova AR, Khafizova IF, *et al*. CCL27: Novel cytokine with potential role in pathogenesis of multiple sclerosis. *Biomed Res Int* 2015;2015:189638. doi: 10.1155/2015/189638.
38. Lindahl H, Guerreiro-Cacais AO, Bedri SK, *et al*. IL-22 binding protein promotes the disease process in multiple sclerosis. *J Immunol* 2019;203(4):888-98. doi: 10.4049/jimmunol.1900400.
39. Kreymborg K, Etzensperger R, Dumoutier L, *et al*. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. *J Immunol* 2007;179(12):8098-104. doi: 10.4049/jimmunol.179.12.8098.
40. Perriard G, Mathias A, Enz L, Canales M, *et al*. Interleukin-22 is increased in multiple sclerosis patients and targets astrocytes. *J Neuroinflammation* 2015;12:119. doi: 10.1186/s12974-015-0335-3.
41. Wing AC, Hygino J, Ferreira TB, *et al*. Interleukin-17- and interleukin-22-secreting myelin-specific CD4(+) T cells resistant to corticoids are related with active brain lesions in multiple sclerosis patients.

Immunology 2016;147(2):212-20. doi: 10.1111/imm.12552.
42. Almolda B, Costa M, Montoya M, González B, Castellano B. Increase in Th17 and T-reg lymphocytes and decrease of IL22 correlate with the recovery phase of acute EAE in rat. *PLoS One* 2011;6(11):e27473. doi: 10.1371/journal.pone.0027473.
43. Muls N, Dang HA, Sindic CJ, van Pesch V. Fingolimod increases CD39-expressing regulatory T cells in multiple sclerosis patients. *PLoS One* 2014;9(11):e113025. doi: 10.1371/journal.pone.0113025.
44. Kürtüncü M, Yılmaz V, Akçay Hİ, *et al*. Impact of fingolimod on CD4+ T cell subset and cytokine profile of relapsing remitting multiple sclerosis patients. *Neuroimmunol* 2019;337:577065. doi: 10.1016/j.jneuroim.2019.577065.
45. Abdel-Dayem MA, Shaker ME, Gameil NM. Impact of interferon β-1b, interferon β-1a and fingolimod therapies on serum interleukins-22, 32α and 34 concentrations in patients with relapsing-remitting multiple sclerosis. *J Neuroimmunol* 2019;337:577062. doi: 10.1016/j.jneuroim.2019.577062.
46. Rosso M, Chitnis T. Association between cigarette smoking and multiple sclerosis: A review. *JAMA Neurol* 2020;77(2):245-53. doi: 10.1001/jamaneurol.2019.4271.
47. Correale J, Farez MF. Smoking worsens multiple sclerosis prognosis: two different pathways are involved. *J Neuroimmunol* 2015;281:23-34. doi: 10.1016/j.jneuroim.2015.03.006.