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Evaluation of the Relationship between Proinflammatory Cytokine Levels and Clinical Findings of Fibromyalgia Syndrome

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ABSTRACT

Background: Immune system has an important effect on pain-related disorders such as fibromyalgia syndrome (FMS). There is no specific laboratory technique for the diagnosis of FMS, but measuring serum proinflammatory cytokines may help.

Objective: The purpose of our study was to determine the serum levels of immune mediators and their relationship with FMS symptoms.

Methods: 25 healthy individuals and 29 FMS patients receiving pregabalin 150 mg/day for a minimum of 3 months were included in this study. FMS patients were diagnosed according to diagnostic criteria of the American College of Rheumatology (ACR 2010). Widespread pain index (WSI), fatigue, waking unrefreshed, cognitive symptoms, somatic symptoms, and Fibromyalgia Impact Questionnaire (FIQ) scores were evaluated in patients with FMS. Serum levels of proinflammatory cytokines (IL-2, IL-6, IL-12, IL-17, IFN-γ, TNF-α) were assessed using enzyme-linked immunosorbent assay (ELISA).

Results: Proinflammatory cytokine levels were higher in the control group than patients with FMS ($P<0.05$). A positive correlation was found between age and WSI ($P=0.037$). In addition, a significant positive relationship was determined between IL-17 level and waking unrefreshed ($P=0.049$). There was no significant relationship between other cytokines and clinical findings.

Conclusion: Lower proinflammatory cytokine levels identified in FMS patients may be related to pregabalin treatment, and there may be an impairment in the inflammatory response. On the contrary, IL-17 showed positive correlation with waking unrefreshed.

Keywords: Cytokines, Fibromyalgia syndrome, Proinflammatory, Symptoms

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INTRODUCTION

The FMS is a common disease including various symptoms such as widespread pain, anxiety, sleep disturbance, and depression (1). The FMS frequently affects women between the ages of 30-50, and the prevalence is between 1-4% of the whole population in studies, and it varies between 0-4% in men and 2.5-10.5% in women (2-4). The disease, which is accompanied by symptoms such as diffuse body pain (hyperalgesia and /or allodynia), tenderness in certain anatomical areas (sensitive spots), chronic fatigue, sleep disturbance, stiffness, and subjective swelling, disrupts the quality of life of the person due to both physical and psychological disorders (5) The etiology in fibromyalgia has not been properly comprehended. It is thought that many mechanisms contribute to the development of the FMS (6). Evidence of some biochemical, neurohormonal, immunological, psychological, and environmental factors that may have an important impact on the disease have been found (7). The immune system mediators, acting as proinflammatory and anti-inflammatory are used to clarify the important mechanisms of many inflammatory diseases. Recent studies have shown that cytokines, the most important mediators of the immune system, may have a key function in the FMS. It has been suggested that the balance between pro and anti-inflammatory cytokines is disrupted in favor of proinflammatory cytokines in fibromyalgia (8, 9). However, different results were obtained in relevant studies in this field. Increasing levels of proinflammatory cytokines (such as IL-6, IL-1β) in the FMS were reported in several studies (10-13). Mandieta et al. measured the levels of inflammatory mediators in the FMS patients and found out that the levels of IL-6 and IL-8 were higher than in the healthy volunteers. They reported that the levels of IL-6 and IL-8 correlate with the severity of fibromyalgia symptoms and cytokines; IL-6 and IL-8 could have additive effects on the chronic widespread pain in fibromyalgia (10). On the other hand, Ranzolin et al. could not find any differences in cytokine concentration between the FMS patients and the healthy individuals. No significant correlation was found between cytokines and disease severity (14). However, in recent studies, it has been suggested that possible failures in the regulation of proinflammatory cytokines may also be related to disease symptoms., such as pain attitude, depression, anxiety, and other neurologically related effects (15, 16).

Therefore, our study aims to detect serum concentrations of proinflammatory cytokines in the FMS patients and the controls and determine their association with disease symptoms. Additionally, the correlation between cytokine levels and clinical findings of the FMS was evaluated with the FIQ. This is the first clinical study that measured serum proinflammatory cytokine levels using ELISA method among subjects with the FMS and a healthy control group.

MATERIAL AND METHODS

Patients

This study has included a total of 54 volunteers, 29 of whom were women with the FMS and 25 were healthy women. Those with the FMS used 150 mg PGB per day for the last 3 months and the patients were collected from the physical medicine and rehabilitation outpatient clinic. Patients were evaluated using 2010 ACR diagnostic criteria and the scoring was done using FIQ (17, 18). Inclusion criteria of patients were that they were diagnosed with the FMS in the last 2 to 3 years and the symptoms were following the ACR diagnostic criteria. Exclusion criteria were acute infectious diseases, autoimmune, metabolic, allergic, or chronic inflammatory disease, and pregnancy. The study protocol was reviewed and approved by the Ethics Committee of the University and was granted by the Scientific Investigation Unit of the Faculty (Project number: KUAP(T)-2020/2).

Sample Collection

The venous blood was taken from the FMS patients and healthy individuals in the morning and 20 ml blood samples were collected in sterile tubes. The serum was obtained by centrifuging the blood samples at 3.000 ×*g* for 10 minutes and samples were stored at (-80ºC) until the analyzing date.

Measurement of the Serum Cytokine Levels

Serum levels of proinflammatory cytokines (IL-2, IL-6, IL-12, IL-17, IFN-y, and TNF-α) were measured using the ELISA kit (BT-LAB, Shangai, China) and studied under the instructions for use. Briefly, the serum samples were added to the wells of plates pre-coated with human IL-2, IL-6, IL-12, IL-17, IFN-γ and, TNF-α antibodies. And then biotinylated human antibodies were added and bound to antigens in the samples. Streptavidin-HRP is added and bound to the biotinylated antibodies. After incubation unbound, Streptavidin-HRP is washed away and substrate solution is added. The intensity in the color of the wells varied in proportion to the amount of human IL-2, IL-6, IL-12, IL-17, IFN-γ , and TNF-α. In the last step, the stop solution is added and the plate is read at 450nm. All samples were measured twice and cytokine concentrations were calculated using standard curves. The validated detection limits were 2.51 ng/L for IL-2, 1.03 ng/L for IL-6, 0.13 ng/L for IL-12, for IL-17, 1.06 ng/L, for IFN-γ, 0.49 ng/L and 1.52 ng/L for TNF-α.

Statistical Analysis

The power analysis of the study was obtained based on the data found by Behm et al. (19). Sample size calculation was made according to the mean (±sd) "IL-6" value. In a total of 201 participants, the "IL-6" values were found to be 2799 (±4182) in the control group (n=91) and 276 (±437) in the patient group (n=110). Using the large effect size (d=0.85), a total of 46 participants were estimated for the power of 0.80 and the alpha power of 0.05. The power analysis was done with GPower 3.1 (http://www.gpower.hhu.de/) version. The Shapiro-Wilk test was employed to determine whether the variables followed a normal distribution. Continuous variables are shown as median (IQR) and mean±standard deviation. The Mann-Whitney U test was performed to compare the two groups based on the normality-test findings. The correlations between continuous variables were analyzed by correlation analysis, and the Spearman correlation coefficients were calculated. SPSS (IBM Corp. Published 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) was used for statistical analysis and reported a statistically significant P<0.05.

RESULTS

No statistically significant difference was observed between the healthy control group and the FMS patients in this study in terms of the mean age (P=0.183).

Table 1 shows laboratory variables;

Table 1. Comparison of healthy controls and FMS patients

	Healthy Controls (n=25)	FMS Patients (n=29)	P value[a]
IL-2	10,17(4,76)	8,90(1,25)	0,001
IL-6	82,39(39,67)	74,84(13,95)	0,049
IL-12	250,57(118,72)	218,58(32,98)	0,006
IL-17	79,33(27,24)	69,16(6,42)	0,001
IFN-γ	47,07(20,43)	43,06(7,85)	0,004
TNF-α	108,61(51,27)	87,14(13,40)	<0,001

FMS: Fibromyalgia Syndrome,, IL: Interleukin, IFN-*γ*: Interferon gamma, TNF-*α*: Tumor necrosis factor alpha; Data was presented as median(IQR). [a]:Mann-Whitney U Test; P<0.05

proinflammatory cytokines in the FMS patients and in the healthy controls. Proinflammatory cytokine levels in the healthy controls were also remarkably higher than in the FMS patients (P<0.005) (Table 1). Especially IL-2, IL-17, and TNF-α showed higher coefficient of the difference between the groups (P=0.001, P=0.001, P<0,001, respectively) (Figures 1-3) and IL-6, IL-12, IFN-γ were observed lower (P=0.049, P=0.006, P=0.004, respectively) (Figures 4-6).

In Table 2 , it is shown that there is a relationship between the serum levels of proinflammatory cytokines and the prominent symptoms of patients. A remarkable association was detected between the WSI total score and the age (P=0.037). WSI score increased with age. In addition, IL-17 concentration was positively correlated with scores of waking unrefreshed (P=0.049). There was no remarkable difference between the levels of proinflammatory cytokines and the reported symptoms.

DISCUSSION

It has been reported in the previous studies that the FMS and similar chronic pain syndromes work in a complex way and need a multi-systemic approach. Immunological, endocrinological, and neural mechanisms all

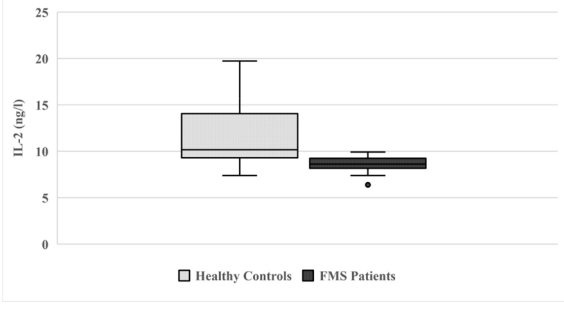

Figure 1. IL-2 levels of healthy controls and FMS patients

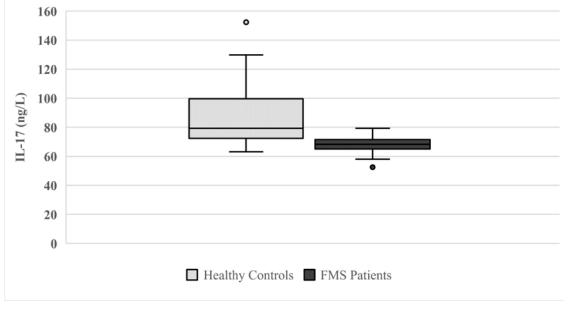

Figure 2. IL-17 levels of healthy controls and FMS patients.

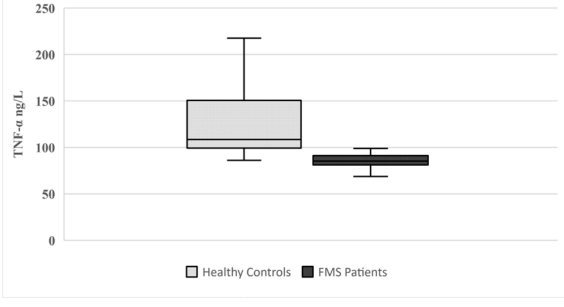

Figure 3. TNF-α levels of healthy controls and FMS patients.

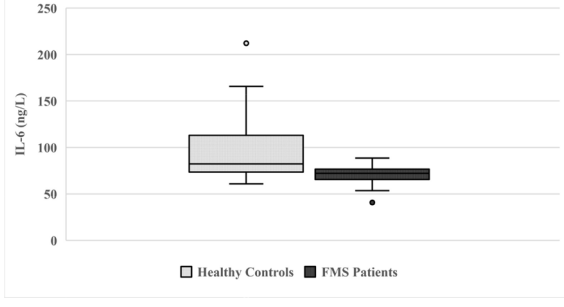

Figure 4. IL-6 levels of healthy controls and FMS patients.

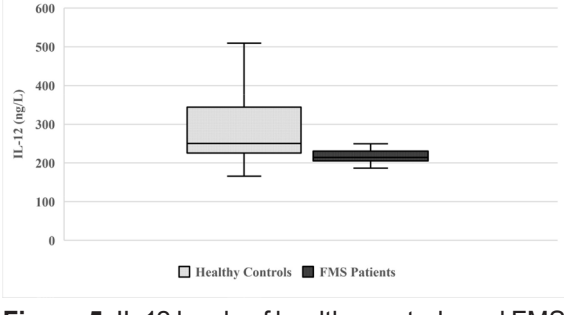

Figure 5. IL-12 levels of healthy controls and FMS patients.

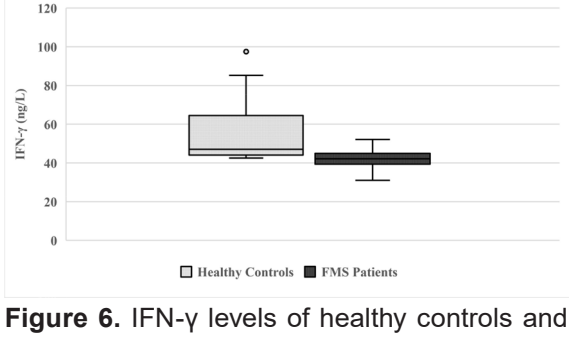

Figure 6. IFN-γ levels of healthy controls and FMS patients.

Table 2. The relationship between clinical findings with age and pro-inflammatory cytokines

(n=29)	WPI		Fatigue		Waking Unfreshed		Cognitive Symptoms		Somatic Symptoms		Sypmtom Severity		FIQ Total score	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
Age	0,39	**0,037**	-0,11	0,574	0,10	0,608	0,06	0,774	0,08	0,694	0,00	0,997	0,29	0,133
IL-2	0,09	0,637	-0,12	0,537	-0,03	0,882	0,15	0,451	-0,26	0,175	-0,04	0,851	0,05	0,809
IL-6	0,01	0,950	-0,13	0,501	0,09	0,656	0,09	0,629	-0,07	0,731	0,04	0,845	-0,23	0,230
IL-12	0,07	0,732	0,04	0,838	0,21	0,277	0,03	0,896	0,07	0,724	0,14	0,474	-0,34	0,071
IL-17	0,11	0,582	0,12	0,538	0,37	0,049	-0,06	0,748	-0,17	0,369	0,09	0,626	-0,32	0,092
IFN-γ	0,07	0,712	0,13	0,515	0,31	0,098	-0,07	0,712	0,17	0,370	0,17	0,379	-0,25	0,185
TNF-α	-0,01	0,978	0,02	0,929	0,04	0,853	0,05	0,790	-0,15	0,431	0,03	0,890	-0,17	0,383

FMS: Fibromyalgia Syndrome,, IL: Interleukin, IFN-*γ*: Interferon gamma, TNF-*α*: Tumor necrosis factor alpha, WPI: Widespread pain intensity, FIQ: Fibromyalgia impact questionnaire; r_s: Spearman correlation coefficient; P<0.05

have some impact on the disease's course. In our study, the relationship between cytokines, and the clinical findings of the FMS was investigated. The results suggest that IL-17 cytokine may affect the development and maintenance of the FMS.

Proinflammatory cytokines have been investigated in previous studies on the FMS (20). Complaints such as fatigue, muscle, and joint pain, depression, anxiety, sleep disorders, somatic and cognitive dysfunctions are the main findings that appear in the FMS and lower the patient's overall quality of life (21). A study was carried out to investigate the behaviors that occur during the inflammation, and it was observed that there were some behavioral and psychological changes in the patients. This suggests that adaptive responses can control the homeostasis in the body during the inflammation (22). In another study, symptomatic and cellular changes in the brain were demonstrated. Until the stimulation stage, the immune system secretes proinflammatory cytokines, transmitting messages to the brain and regulating behavior. It has been reported that the administration of several immune mediators (IL-6, TNF-α) to animals by central injection may trigger disease symptoms (23).

The presence of various psychological and physical complaints in the FMS patients suggests that this disease may be related to the immune mediators, as well as the nervous system and proinflammatory cytokines may have a key role in the etiopathogenesis of the FMS. In our study, proinflammatory cytokine concentrations of the FMS patients were reported lower than in the healthy controls. In this study, there was no association between the clinical findings and cytokine levels except that IL-17. IL-17 concentrations were lower in the FMS patients but a significant positive relationship was found between the waking unrefreshed.

Correlating with our results in a study investigating cytokine levels in the FMS patients, it has been reported that IL-2 and IFN-γ levels are lower in the FMS patients than in the healthy individuals, and there was no significant relationship between clinical findings and cytokines. (24). Wallace et al. suggested that IL-6 can play an important role in several symptoms of the FMS (25) but we found out that IL-6 concentrations were also low in the FMS individuals and no significant linking was found between the clinical symptoms and IL-6. However, some research found no remarkable difference in serum IL-6 concentrations (26). In another study, IL-6 level was reported higher in the FMS patients (27). On the other hand, there are not many studies on the effectiveness of IL-12 level in the FMS. In this study, it was observed that the IL-12 level was released less in the FMS patients compared to the healthy individuals. In addition, no significant difference was found between clinical findings and IL-12 level. However, there are

contradictory results regarding the role of another proinflammatory cytokine TNF-α in inflammatory diseases in studies conducted to determine the role of the TNF-α in FMS. Various results were obtained, suggesting that the level of the TNF-α was higher (27) or lower (28) in the patient group than in the healthy individuals or no change was reported (29) in the healthy and the patient groups. In our study, the TNF-α releasing was higher in the healthy individuals and there was no association with clinical findings. IL-17 is a less studied proinflammatory cytokine than the others, so there is limited information about IL-17 concerning the FMS. However, in some recent studies, increasing levels of IL-17 in the FMS patients were obtained (30).

On the other hand, it is known that there are important alterations in the immune system in the pathogenesis of chronic pain conditions. Central sensitization is a mean of the immunological state of neuropathic pain with cytokine overexpression and microglial activation. Recent studies have emphasized the effectiveness of cytokines for the management of these pathophysiological conditions which decrease the excitatory transmitter release and neuronal sensitization (31). Pregabalin (PGB) is one of the new generation antiepileptic drugs used in several pain conditions. It works by binding the to voltage-gated potassium and calcium channels and has an antinociceptive effect in neuropathic pain (31). There is limited information on the immunomodulatory activity of PGB on cytokine release. However, in a study with mice evaluating the efficacy of the PGB on cytokine release, it was observed that proinflammatory (TNF-α, IL-1β, IL-6, and IL-2) cytokines were inhibited by PGB (31). Likewise, Kilic et al. reported that all doses of pregabalin had anti-inflammatory effects supported by cytokine levels in adult female rats. They found that PGB significantly decreased the IL-1β and TNF-α levels (32). In this study, a noticeable reduction in all proinflammatory cytokines was detected in the FMS patients using PGB. Unlike previous studies, proinflammatory cytokines were found to be lower in the FMS patients in our study, and we suggest that PGB may have a suppressive effect on these cytokines. On the other hand, although PGB was employed, the positive correlation between IL-17 level and waking unrefreshed in the FMS patients indicates that this cytokine may play a crucial role in the development of fibromyalgia.

CONCLUSION

Consequently, in our study, the concentration of IL-17 was observed to correlate positively with waking unrefreshed, and waking unrefreshed is a common finding of the FMS associated with neurological pathways. So, our findings indicate the accuracy of the hypotheses that some of the cytokines may play a key role in the development of neuropathic pain symptoms. On the other hand, contrary to expectations, low proinflammatory cytokine levels were detected in the FMS patients, which may be the consequence of the anti-cytokine effects of long-term pregabalin treatment. There have been a few researches on this subject, but more are needed to reach a firm opinion on how the PGB affects cytokine levels in patients with the FMS.

Some drawbacks of this study are the small number of patients and the lack of male participants. The roles of other cytokines in these patients can be investigated in future studies. Further studies should investigate the relationship between cytokines and their correlations with clinical symptoms in fibromyalgia.

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Conflicts of Interest: None declared.

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