Research Paper　　　　　　　　　　　　**Medical Science**

Serum Interleukin-23 Levels And Relationship With Clinical Symptoms of Fibromyalgia Syndrome

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ABSTRACT

30 premenopausal female patients with fibromyalgia syndrome diagnosed according to ACR 1990 criteria and 35 premenopausal healthy females were included in this study. Serum interleukin-23 levels were measured in the sera of both the patient and control groups. Widespread body pain, headache, fatigue, morning stiffness, sleep disorder, the number of tender points, fibromyalgia impact questionnaire and depression were evaluated as clinical parameters in the patients with fibromiyalgia syndrome. The mean serum interleukin-23 levels of patients with fibromiyalgia syndrome and control groups were determined as 236.44±116.88 pg/ml and 130.27±73.23 pg/ml, respectively, and there was significant difference between the groups. There was no significant correlation between serum interleukin-23 levels and most of the clinical symptoms of fibromiyalgia syndrome.Although we determined higher serum IL-23 levels in fibromiyalgia syndrome patients compared to healthy controls, it was concluded that serum interleukin-23 levels may not be related to the clinical symptoms of fibromiyalgia syndrome.

KEYWORDS | Fibromiyalgia syndrome, interleukin-23, clinical symptoms

Introduction

Fibromyalgia syndrome (FMS) is a chronic pain syndrome, the pathogenesis of which is complex and diagnosed with at least 11 out of 18 positive tender points according to the 1990 criteria of the American College of Rheumatology (ACR) [1]. Additional symptoms include depressive mood, fatigue, and sleep disturbance. Although numerous studies have been conducted to explain the pathophysiology of pain, it remains unclear. To date, neuroendocrine mechanisms, various hormones, serotonin, melatonin, substance P, endorphins, and vitamin D have been researched in an attempt to explain the pathophysiology of FMS [2-5].

Of the many theories concerning the pathophysiology of FMS, one considerable hypothesis argues that aberrations of the hypothalamic-pituitary-adrenal (HPA) axis which are related to or caused by cytokine imbalance may play a role in the pathophysiology of FMS [6,7]. Cytokines are immunomodulating proteins with a critical function in various biological pathways. Considerable data have shown that cytokines are key players in the induction and maintenance of pain. Some researchers have even suggested that the biochemical origin of all pain is inflammation and the inflammatory response. Pro- and anti-inflammatory cytokines have been extensively investigated in both the etiology of FMS and in the intensity of core symptoms but results have been conflicting [8-12]. Although there are some studies which have reported altered cytokine levels or a relationship of cytokines with core symptoms of FMS, other studies have reported no relationship. The aim of this study was to determine the relationship between IL-23 and FMS and core symptoms of FMS.

Materials and Methods

The study included 30 premenopausal female FMS patients (aged 27-50 years, mean age, 44.1±10.9 years) who were admitted to our outpatient clinic and met the 1990 American College of Rheumatology (ACR) criteria [1] for the diagnosis of FMS. Since neuroendocrine abnormalities have a role in FMS etiopathogenesis, premenopausal women were included in the study. Participants who had neurological, inflammatory, endocrine, chronic disease, osteoporosis, pregnancy or a history of severe psychiatric illness were excluded from the

study. Patients who had quit the usage of any regular drug (including corticosteroids, antidepressants, anticonvulsants, opiates) for 2 weeks prior to testing and usage of analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) on the analysis day were included in the study.

The control group was composed of 35 age-matched healthy premenopausal women who had normal physical examination and routine test results and had no chronic or endocrinological diseases.

Venous blood samples were obtained from FMS patients and the healthy controls between 9 and 11.00 a.m. and serum IL-23 levels were measured. The demographic characteristics, clinical and laboratory findings were recorded by the same researcher. Serum IL-23 levels were measured using human IL-23 enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, Vienna). Immunonephelometric method (Siemens, Munich, Germany) was used to measure serum CRP levels and results were given as mg/L. Capillary photometric method was used to measure ESR and results were given as mm/hour. Widespread body pain, headache, fatigue, morning stiffness, sleep disorder, the number of tender points, Fibromyalgia Impact Questionnaire (FIQ) and Beck depression scores were evaluated as clinical findings in the patients. FIQ was evaluated by the Turkish version [13] of original questionnaire [14]. The number of tender points was determined by ACR 1990 diagnostic criteria [1]. Depression scores were evaluated using the Turkish version [5] of the original Beck Depression Scale [16]. Widespread pain and fatigue were measured with 10-cm visual analog (VAS) scale [17]. Morning stiffness was evaluated by the morning stiffness scale of the Lequesne index [18] (0=no stiffness, 1=low stiffness for <15 minutes, 2=severe stiffness for >15 minutes). Sleep disorder was evaluated with the sleep latency component of the Pittsburgh Sleep Quality Index [19] (0= no difficulty or difficulty falling asleep for ≤ 15 minutes, 1=difficulty falling asleep for 16-30 minutes, 2=difficulty falling asleep for 31-60 minutes, 3=difficulty falling asleep for >60 minutes). Headache was evaluated using 4 definitive words of the Short-Form McGill Pain Questionnaire used to determine the affective dimensions of pain [20] (0=no headache, 1=low level headache, 2=mild headache, 3=severe headache).

Data were analyzed using the SPSS/PC statistical software package (SPSS, v.20.0 for Windows, SPSS Inc. Chicago). Descriptive statistical methods (mean, standard deviation) were used to evaluate the data. All results were expressed as mean±standard deviation and percentage. The unpaired Student's t test was used to evaluate the significance of differences between the groups. Spearman correlation analysis was used to determine the correlations between findings. A values of p<0.05 was considered statistically significant, at 95% confidence interval.

This study was approved by the local ethics committee, and informed consent was obtained from each participant and patient anonymity was preserved. The procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation and with the Helsinki Declaration of 1975, as revised in 2002.

Results

There was no significant difference between the demographic characteristics of the FMS patients and the healthy controls (p=0.38). The mean disease duration of FMS patients was 8.9±6.7 years. The mean serum IL-23 levels in the patient and control groups were 236.44±116.88 pg/ml and 130.27±73.23 pg/ml, respectively, and there was significant difference between the groups (p<0.001) (Table 1).

Table 1. Demographic and laboratory data of groups

	FMS Group (n=30)	Control Group (n=35)	p
Age (years, mean±SD)	44.1±10.9	42.4±10.7	0.38
IL-23 (pg/ml)	236.44±116.88	130.27±73.23	<0.001
CRP (mg/dl)	0.85±0.40	0.57±0.40	0.07
ESR (mm/h)	20.25±9.57	15.15±4.53	0.07

IL-23: Interleukin-23, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, FMS: Fibromyalgia syndrome, SD: Standard deviation, p: test statistic p value

Widespread body pain, headache, fatigue, morning stiffness, sleep disorder, the number of tender points, FIQ and depression were evaluated as clinical parameters in the FMS patients. The mean, standard deviation and percentage values of the clinical findings are shown in Table 2.

Table 2. Mean, standard deviation and percentage values of clinical symptoms in FMS patients

Clinical symptom	Frequencies
WBP	6.10±1.88
Fatigue	6.20±1.65
FIQ	33.50±17.02
NTP	10.53±3.19
Beck DS	16.03±5.81
Morning Stiffness (minutes)	7.67±5.68
Headache	70.00%
Sleep Disorder	66.60%

SD: Standart deviation, WBP: Widespread Body Pain, FIQ: Fibromyalgia Impact Questionnaire, NTP: Number of Tender Points, Beck DS: Beck Depression Scores. Results are shown as mean±SD for WBP, fatigue, FIQ, NTP, Beck DS and morning stiffness; as percentage of patients having clinical symptom for headache and sleep disorder.

IL-23 levels were positively and significantly correlated with CRP (r=0.516, p=0.004) and ESR (r=0.850, p<0.001in the patient group.

When correlation analysis was performed to evaluate the relationship between IL-23 and the clinical parameters of FMS, a significant correlation was found only between IL-23 and morning stiffness (p<0.001). No significant correlation was found between IL-23 levels and the other parameters except morning stiffness (p>0.05 for all parameter) (Table 3).

Table 3. Correlations serum IL-23 levels and clinical symptoms in FMS patients

		WBP	Fatigue	FIQ	NTP	Beck DS	Morning Stiffness	Headache	Sleep Disorder
IL-23	(r)	-.204	.205	.167	.085	.192	.750**	.090	.147
	(p)	0.279	0.277	0.367	0.656	0.308	<0.001	0.636	0.438

r: correlation coefficient, p: test statistic p value, WBP: Widespread Body Pain, FIQ: Fibromyalgia Impact Questionnaire, NTP: Number of Tender Points, Beck DS: Beck Depression Scores

Discussion

FMS is a chronic pain syndrome, the pathogenesis of which is complex and incompletely understood. Pain, fatigue and distressed mood are the most prominent symptoms of FMS. Considering that there are no specific diagnostic laboratory tests of FMS [21], research and identification of the biomarkers of the syndrome would be very helpful in the diagnosis of symptoms. In this study we aimed to analyze serum IL-23 levels in FMS. To the best of our knowledge, this the first study investigating serum IL-23 levels in FMS. Our results showed that the mean serum IL-23 levels were significantly higher in patient group compared to control group (236.44±116.88 pg/ml and 130.27±73.23 pg/ml, respectively). However, IL-23 levels were not significantly related with the clinical parameters, except morning stiffness. IL-23 is a heterodimeric cytokine

with a specific p19 subunit and a p40 subunit shared with IL-12 which has additional inflammatory effects apart from IL-12 [22]. Previous studies have demonstrated that IL-23 can increase and stabilize the Th17 cells in disease models and humans and it has been suggested that IL-23 plays the main role in some inflammatory diseases [22].

Increased levels of cytokines and key inflammatory mediators such as C-reactive protein (CRP), have been reported to be related with symptoms of pain, fatigue, and distressed mood in multiple conditions including fibromyalgia and chronic widespread pain [23]. The symptoms are thought to arise from a combination of interactions between the autonomic central nervous system [24], the hypothalamic–pituitary–adrenal (HPA) axis [25] and the immune system [26]. Therefore, fibromyalgia can not be considered to be an inflammatory disease but it is clear that the syndrome has an immunological component [8].

Supporting this opinion, pro- and anti-inflammatory cytokines have been extensively investigated in both the etiology of FMS and in the intensity of core symptoms but results have been conflicting. There have been studies reporting high [10], normal [8] and unaltered [9] serum or plasma cytokine levels. There have also been studies which have reported increased [8], normal [27] and decreased [12] production of inflammatory cytokines in the stimulated or unstimulated culture supernatants of peripheral blood mononuclear cells (PBMC). Moreover, cytokine levels produced by cultured mononuclear cells have been reported even to show discrepancy in stimulated or unstimulated conditions [8].

Although there is a large number of studies available in literature, different methods of analysis, different biological materials and different sample sizes make a direct comparison of results impossible . In a systematic review and meta-analysis which set out to clarify various cytokine profiles in FMS, only IL-6 levels were found to be higher in patients with FMS compared to healthy controls [28]. Futhermore, studies researching the relationship of cytokine levels with the symptoms of FMS have produced conflicting results with an inconsistent pattern [8,11,12,29].

The relationship between pain, the most prominent symptom of FMS, and cytokine levels has been widely investigated and cytokines have been reported to be related with inflammatory and neuropathic pain [30]. IL-6 and IL-8 have been reported to be responsible for hyperalgesia of FMS patients [8]. These findings suggested that IL-23 may have similar effects but in the current study, no relationship was determined between IL-23 and the most of clinical parameters of FMS except morning stiffness. However, the role of cytokines in FMS can not be completely excluded, as some neuropsychiatric symptoms of FMS have been shown to be more related with disordered cytokine production of some tissues such as glial cells than circulating cytokines [31].

There are some limitations in this research as in other cytokine level studies in literature. The results of the current study demonstrated a wide range of IL-23 levels in FMS patients with a large standard deviation (177.50 pg/ml). Large standard deviations for cytokine values reflecting inherent biological variability is a prominent limitation of cytokine analysis in clinical research [32]. This has also been observed in many other studies conducted on patiens with chronic diseases [33]. It has been speculated that stress level, diurnal variation, exercise, dietary intake and medications may be responsible for large variations between subjects [34]. In addition, researchers have emphasised that FMS is complex disorder with a wide variation in phenotypic presentation and this might also explain the wide variations of cytokine levels [32]. Another factor in the different results of cytokine levels in FMS patients may be differences of disease duration between patients. In a study conducted to explore IL-6 and IL-8 levels in FMS patients, no significant difference was reported in terms of cytokine levels between patients diagnosed as FMS for less than 2 years and

healthy controls [8]. The researchers suggested that this was due to chronic subclinic inflammation and the lack of inflammatory response. May be the non-significant correlations observed between IL-23 and the symptoms other than morning stiffness in our study may be attributed to the subclinic inflammation of our patients having a disease duration of approximately 8 years.-

There are other limitations in cytokine level researchs. It is well known that an inflammatory profile may show individual variations and may show alterations in the same person at different times. One description for the inconsistency of cytokine level researchs is the short half-life of cytokines in cytokine-bound receptors which are undetected in blood work results [23,35]. Taking into consideration that evaluating circulating cytokine levels ignores cellular cytokine receptor levels and cytokine receptor antagonist levels which may affect the amount of circulating cytokines, further studies analyzing cellular cytokine receptor expression and cytokine receptor antagonists together with circulating cytokine levels would be more credible.

In conclusion, evaluating the conflicting results in literature and those of the current study, cytokines are considered to be only one component of FMS paradigms and cytokine changes are considered not to be causative but secondary to other factors [9]. Similar to this opinion, it can be speculated that cytokine changes in FMS are not likely to be a dominant factor on the core symptoms of FMS. To the best of our knowledge this is the first study examining IL-23 levels and the relationship with clinical symptoms of FMS patients. Although the small sample size is a limitation of our preliminary study, it can be considered valuable guidance for further research to clarify the role of cytokines in the pathogenesis of FMS.

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