Cytokines and Depression in Cases with Fibromyalgia

ALI GÜR, MEHMET KARAKOÇ, KEMAL NAS, REMZI ÇEVIK, AZIZ DENLI, and JALE SARAÇ

ABSTRACT. ***Objective.*** Fibromyalgia (FM) is a chronic, painful musculoskeletal disorder characterized by widespread pain, pressure, hyperalgesia, morning stiffness, and an increased incidence of depressive symptoms. The etiology, however, has remained elusive. The aim of the present study was to examine the inflammatory response system in FM and to investigate the effect of depression level on serum cytokines.

Methods. Serum interleukin-1 (IL-1), IL-2 receptor (IL-2r), IL-6, and IL-8 and the Hamilton Depression Rating Scale (HDRS) score were determined in 32 healthy volunteers and in 81 patients with FM, classified according to the American College of Rheumatology criteria.

Results. In our study, serum IL-1 and IL-6 were not statistically significant, but serum IL-8, IL2r, and HDRS score were significantly higher in patients with FM than the control group ($p < 0.01$). In addition, in patients with FM, IL-8 was found to be related to pain intensity ($r = 0.35$; $p < 0.01$).

Conclusion. IL-8 may play an important role in the occurrence of pain in FM. (J Rheumatol 2002;29:358–61)

Key Indexing Terms:
CYTOKINES DEPRESSION FIBROMYALGIA

Fibromyalgia (FM) is a disorder or condition characterized by widespread, chronic musculoskeletal aching and stiffness and pressure hyperalgesia at characteristic sites, called soft tissue tender points[1,2]. The etiology of FM, however, has remained elusive and the treatment remains mainly empirical[3].

Some current etiologic hypotheses are that FM is a rheumatoid-like disease or a disorder of muscular abnormality or repair; that it results from aberrant mechanisms of peripheral pain; that it is a psychoneuro-endocrine-immune disorder, a psychomatic disorder, or a psychiatric disorder related to major depression[4,5]. There are, however, few studies that examine the inflammatory response system in FM. It has been suggested that a subgroup of FM patients suffer from a low grade inflammatory process[6] or from exaggerated neurogenic inflammatory responses[7]. But there are few studies that fail to show some activation of cell-mediated immunity[8].

Proinflammatory cytokines, such as interleukin 1 (IL-1) and IL-6, may induce hyperalgesia, for example, by acting on the forebrain tissue surrounding the lateral and third ventricle and may directly influence the responsiveness of nociceptive neurons[9-12]. In experimental animals or humans, IL-1 and IL-6 may induce not only (inflammatory) hyperalgesia but also other symptoms characteristic of FM, such as fatigue, sleep disorders and depression-like symptoms[13,14].

We investigated signs of an activated inflammatory response in FM and significant positive correlations between inflammatory response markers and severity of FM symptoms, such as hyperalgesia, anergy, sleep disorders, and depressive symptoms based on the following: (1) FM may be an inflammatory disorder; (2) proinflammatory cytokines may induce the characteristic symptoms of FM. More specifically, since increased serum concentrations of IL-6, soluble IL-6 receptor (sIL6r), sIL-1r antagonist (sIL-1RA), and sCD8 have been found in patients with inflammatory disorders and major depression[15], we expected to find the same alterations in FM patients[2].

The aim of the present study was to examine the inflammatory response system in FM. Serum IL-1ß, IL-2r, IL-6, and IL-8, and Hamilton Depression Rating Scale (HDRS) score were determined in 32 healthy volunteers and in 81 FM patients, classified according to the American College of Rheumatology (ACR) criteria.

MATERIALS AND METHODS

A total of 113 subjects participated in the present study, i.e., 32 healthy volunteers and 81 patients with FM, recruited from the Department of Physical Therapy and Rehabilitation, University Hospital of Dicle, Diyarbakir, Turkey. FM patients fulfilled the ACR criteria for FM[1]. These criteria include: (a) a history of widespread pain of at least 3 months, i.e., pain in the left side of the body, pain in the right side of the body, pain above and below the waist, axial skeletal pain (cervical spine, anterior chest, thoracic spine, low back pain); and (b) the presence of at least 11 tender point sites (measurements performed using a digital pressure device with a force of 4 kg) that included: occiput L or R, low cervical L or R, trapezius L or R, supraspinatus L or R, second rib L or R, lateral epicondyle L or R, gluteal L or R, greater trochanter L or R and knee L or R.

From the Department of Physical Medicine and Rehabilitation, School of Medicine, Dicle University, Diyarbakir, Turkey.

A. Gür, MD, Associate Professor; M. Karakoç, MD, Resident; K. Nas, MD, Associate Professor; R. Çevik, Associate Professor; A. Denli, MD, Resident; J. Saraç, MD, Professor, Chair, Physical Medicine and Rehabilitation, School of Medicine.

Address reprint requests to Dr. A. Gür, Physical Medicine and Rehabilitation, Dicle University School of Medicine, Diyarbakir, Turkey. E-mail:alig@dicle.edu.tr

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Major clinical conditions other than FM were excluded by physical examination and laboratory investigations of routine blood cells and differentials, red blood cells, hematocrit and hemoglobulin, baseline thyroid-stimulating hormone, and antinuclear autoantibodies.

Exclusionary criteria for FM patients and healthy controls were: (a) recent or past history of psychiatric disorders, e.g., major depressive disorder, alcohol dependence, substance abuse, schizophrenic or paranoid disorder, personality disorder, and somatoform disorder; (b) immunocompromised subjects; (c) subjects with neurological, inflammatory, endocrine or clinically significant chronic disease, such as diabetes mellitus, rheumatoid arthritis, inflammatory bowel disease, and organic brain disorders; (c) abnormal liver function tests, such as serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transpeptidase; and (d) pregnant females.

All subjects were free of infection, inflammatory or allergic reactions for at least 2 weeks prior to blood sampling and free of drugs known to affect immune or endocrine functions and of hormonal preparations. None of the 32 healthy volunteers fulfilled ACR criteria for FM and none was a regular drinker or had ever taken psychotropic drugs. Each patient had normal findings on radiography of the chest, hands, feet, and sacroiliac joints.

After clinical diagnosis of patients, blood samples for routine screening were performed. Every patient was checked for study inclusion and exclusion criteria and underwent a 2 week drug-free period. At the end of this period, each patient underwent a disease-oriented clinical examination (i.e., tender point, morning stiffness, etc. See Table 2) and blood was sampled for the assay of serum IL-1, IL-2r, IL-6, and IL-8 after overnight fast. Blood collections were performed in standardized conditions to minimize sources of preanalytical variation. Serum IL-Iß, IL-6, IL-8, and sIL-2R levels were determined with quantitative ELISA and IMMULITE diagnostic kits (DPC-Diagnostic Products Corporation, USA). We measured IL-Iß (normal range 0–5 pg/ml), IL-6 (normal range 0–5.4 pg/ml), IL-8 (normal range 0–62 pg/ml), and sIL-2R (normal range 223-710 U/ml). The assays have undergone extensive testing with a multitude of different cytokines to ensure absence of cross-reactivity with other molecules.

The pressure was measured at each of the above 18 tender points. The total tender point score (R+L) was used in the statistical analyses. Clinical assessments were carried out the same morning as the blood collections. To assess the association between depressive symptoms in FM and serum cytokines we divided FM patients into those with a HDRS of 16 (indicating substantial depressive symptoms) and a HDRS < 16.

Statistical analysis. Results in patients with FM and controls were compared using Student's unpaired t test. Comparisons of results of interleukins in each group were obtained using analysis of variance. Pearson's correlation test was used for correlation analysis. General characteristics of the sample are described using means (95% confidence intervals) for parametric data. All statistical tests were 2-sided; $p < 0.05$ was considered to be statistically significant. Values are expressed as the mean ± standard deviation.

RESULTS

The mean ages of the 81 patients and 32 controls were 27.61 ± 7.35 and 28.93 ± 6.17 years, respectively; the difference between patients and controls was not statistically significant ($p > 0.05$). In our study, serum IL-1 and IL-6 were not statistically significant, but serum IL-8 and IL-2r were significantly higher in patients with FM than control group ($p < 0.05$) (Table 1). In patients with FM IL-8 was found to be related to pain intensity ($r = 0.35$; $p< 0.01$) (Table 2).

DISCUSSION

FM is a clinical entity of unknown etiology. Diagnostic criteria, now generally adopted, were defined by the American College of Rheumatology in 1990[1]. Generally, the onset of symptoms occurs between the ages of 20 and 40 years, and affected patients are predominantly women. Standard laboratory tests and imaging techniques are nonspecific. Immunoglobulin deposits in skin[16], elevation in the level of serum IL-2[17], FM-like syndrome induced by intravenous IL-2 treatment in patients with neoplasm[18], altered IL-2 production by T helper/inducer lymphocytes[19] and abnormal T cell subpopulations[18,20] have been reported. Potential involvement of the immune system in the pathogenesis of this disorder has been suggested by some authors[21].

Table 1. Measurements of serum interleukin-1ß (IL-1ß), IL-2 receptor (IL-2r), IL-6, and IL-8, and age in healthy volunteers (HV) and patients with fibromyalgia with (+D) and without (–D) Hamilton Depression Rating Scale (HDRS) score ≥ 16.

	All FM (n = 81)	HV (n = 32)	FM–D (n = 37)	FM+D (n = 44)
IL-1ß (pg/ml)	5.14 ± 0.98	5.02 ± 0.13	4.87 ± 0.8	5.35 ± 1.68
VC,%	19	2.5	16	31
IL-2r (U/ml)	527.56 ± 169.65[a]	473.3 ± 174.92	538.6 ± 187.3[a]	499.4 ± 141.3
VC,%	32	36.9	34.7	28.2
IL-6 (pg/ml)	5.52 ± 3.96	5.46 ± 1.378	5.16 ± 0.69	5.01 ± 2.08
VC,%	71	25	13	41
IL-8 (pg/ml)	13.06 ± 8.04[a]	10.12 ± 5.18	14.82 ± 9.84[a]	11.38 ± 4.97
VC,%	61	51	66	43
HDRS score	17.22 ± 5.83[a]	3.08 ± 3.15	12.41 ± 4.15[a]	21.32 ± 3.4[a,b]
VC, %	33	101	33	15.9
Age (yrs)	27.61 ± 7.35	28.93 ± 6.17	26.65 ± 6.84	29.29 ± 7.47
VC,%	26	21	25.6	25.5

Values are mean ± standard deviation for all variables; where no superscript appears, there is no significant difference; VC: coefficient of variation

[a] Statistically significantly different from HV ($p < 0.05$);

[b] Statistically significantly different from FM-D patients ($p < 0.05$).

Table 2. Correlation between serum cytokines and clinical variables in patients with fibromyalgia.

	IL-1ß (pg/ml)	IL-2r (U/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)
Age	–0.02	–0.02	0.20	–0.01
No. of tender points	0.04	0.03	0.19	–0.04
Morning stiffness	–0.03	0.09	–0.06	–0.03
Fatigue	–0.01	0.01	0.15	0.08
Sleep disturbance	0.21	–0.02	–0.11	0.02
Pain	0.14	0.24	–0.02	0.35*
Muscle spasm	0.06	0.14	–0.04	–0.12
Skinfold tenderness	–0.01	0.12	–0.04	–0.04
HDRS score	0.06	–0.04	–0.05	–0.09

*p < 0.01. IL–1β: interleukin-1β; IL-2r: serum IL-2 receptor; HDRS score: Hamilton Depression Rating Scale score.

No conclusive evidence of an underlying cause or pathophysiologic basis for FM exists, although myriad mechanisms have been proposed[22]. It has been suggested that FM has an immunologic component that includes cutaneous deposition of immunoreactants[21]. An elevation in the level of serum IL-2 in patients with FM has been reported by Peter and Wallace[20]. Wallace and Margolin[18] reported that cancer patients undergoing intravenous recombinant IL-2 therapy experienced FM-like symptoms, suggesting a possible role of IL-2 in the development of FM. The 2 reports, one describing acute onset of FM-like symptoms in cancer patients receiving IL-2[18] and the other elevated serum levels of IL-2 in FM patients[20], are consistent with the suggestion that IL-2 may be involved in the symptoms of FM.

There are no obvious signs of immunological disturbances in FM. The occurrence of serum antibodies does not differ significantly in FM compared to a reference group[23], and no abnormal cytokine levels have been found[24]. Littlejohn, *et al*[7] reported an increased neurogenic inflammation in FM. Mast cells produce several cytokines that when activated can act in both a paracrine and an autocrine way. A role for cytokines in FM has also been suggested by Wallace, *et al*[24] although the cytokine levels [IL-1, IL-2, IL-2r, tumor necrosis factor (TNF)] as measured in serum did not differ between 16 patients and controls. Hader, *et al*[19] found a delayed production of IL-2 in patients with FM, which was corrected by the addition of an inducer of protein kinase C activation.

Clinical studies show that more than 75% of patients with FM complain of poor sleep[1,25,26]. The sleep is described as light, unrefreshing, accompanied by generalized stiffness and/or aching, and profound fatigue upon awakening. The pain, fatigue, and unrefreshing sleep of FM tend to persist. IL-1 is believed to be central to the coordination of certain immunologic substances and neuroendocrines in the regulation of the sleep-wake cycle. Krueger and Obal[27] have proposed that the diurnal sleep-wake rhythm is the result of oscillatory mechanisms that involve brain IL-1 and the neurohormones of the hypothalamic pituitary axis. IL-1 induces hypothalamic release of CRF, which in turn induces pituitary release of ACTH. In addition, IL-1 can be produced by brain glia cells in response to serotonin and catecholamine neurotransmitters[5].

IL-6 is particularly important in FM. gp130 is the signal transducer protein for IL-6 and serum sgp130 may inhibit IL-6 signals through membrane-anchored gp130[28,29]. Moreover, gp130 signals are involved not only in the neuroendocrine, immune, and inflammatory responses, but also in neuromuscular and (central) nervous system activity[30]. Maes[2] and coworkers suggest that serum sgp130 is significantly higher in FM patients than in healthy controls and suggest that there is a positive correlation between stiffness and increased serum sgp130. They also suggest that FM may be accompanied by suppression of inflammatory response functions. The measures of IL-6r and gp130 were not performed in our study.

Stress can alter cytokine levels. The immune system responds to stressors by causing certain immune cells to secrete the pro-inflammatory cytokines, IL-1 and IL-6. Both are involved in inflammation, and IL-6 in particular is thought to worsen the symptoms of autoimmune diseases and FM[31]. Plant sterols and sterolins can reduce the stress hormone cortisol and the proinflammatory immune factor, IL-6 and TNF-α. IL-6 and TNF-α are increased in autoimmune disorders, osteoporosis, over exercising, FM, and osteoarthritis. Reduction of this inflammatory agent is key to halting symptoms and pain, which is what plant sterols and sterolins do[32].

Poor nutrition, coupled with too much stress, is a recipe for illness. When we are under stress our body sends out the stress hormone cortisol. This hormone then causes a proinflammatory immune factor IL-6 to be excreted. IL-6 is involved in the exacerbation of autoimmune disorders and other inflammatory conditions such as FM and osteoarthritis. Sterols and sterolins effectively reduce cortisol, and subsequently IL-6, alleviating symptoms associated with these diseases. Given this cortisol-IL-6 connection, stress reduction is of paramount importance to the health of our immune system[32].

The studies on stress and cytokines in FM have mostly focused on IL-1 and IL-6, but there is insufficient knowledge about the relationship with IL-8. It may be claimed in our study that elevation of serum IL-8 was related to stress and pain in FM, but additional, large scaled studies are needed on this subject.

The major finding of our study is that FM patients with and without significant depressive symptoms show differences in cytokines. Thus, FM patients with more mild depressive symptoms (i.e., HDRS score < 16) have significantly higher serum IL-2r and IL-8 than healthy volunteers.

In conclusion, these results suggest that the IL-8 may

play an important role in the occurrence of pain in FM. In addition, our results do not support the correlation between depression level and serum cytokines in patients with fibromyalgia.

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