POSSIBLE ROLE OF CYTOKINES IN FIBROMYALGIA

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ABSTRACT

Aim of Work: To evaluate the possible role of cytokines in fibromyalgia (FM).

Methodology: Levels of interleukin–6 (IL–6), IL-8, and IL–10 were measured with enzyme linked immunosorbent assay techniques in the sera of 20 FM female patients and 10 normal controls. Results were correlated with the clinical manifestations of the disease.

Results: A significant (p < 0.05) increase in both IL-6 and IL-8 serum levels were found in FM patients as compared to controls. But there was no significant (p > 0.05) difference between FM patients and controls regarding serum IL-10 levels. In FM patients, the elevated IL-6 serum level showed a significant positive correlation (r = +0.62) with fatigue severity score; and a significant negative correlation (r = -0.59) with sleep score. While the elevated IL-8 serum level showed a significant positive correlation with the number of tender points (r= +0.68) and pain score(r= +0.54). On the other hand, IL-10 showed no significant correlation with any clinical variable.

Conclusion: IL-6 and IL-8 may play an important role in FM. IL-6 may be related to fatigue and sleep disturbances. IL-8 may be related to pain intensity and number of tender points in patients. Strategies to decrease the levels of cytokines, may constitute novel hopes for treatment of FM patients.

INTRODUCTION

Fibromyalgia syndrome (FM) is a chronic, painful disorder commonly seen in middle aged women (White et al., 1999). The pathogenesis of FM is not known but consideration has been given to genetic predisposition (Yunus et al., 1999), traumatic injury (Culcasure et al., 1993), affective psychopathology (Ahles et al., 1991), Viral infections
(Goldenberg 1989), and immunological mechanisms (Caro, 1989). It is characterized by the presence of widespread musculoskeletal pains for 3 months, tender points at specific anatomical areas, fatigue, and poor sleep. Associated disorders are restless leg syndrome, irritable bowel syndrome, irritable bladder syndrome, cognitive dysfunction, cold intolerance, multiple sensitivities and dizziness recognized as an important clinical problem associated with high levels of functional disability, emotional distress, anxiety and depression (White et al. 1999).

Much research has been carried out in chronic pain in these patients (Salemi et al., 2003). Research suggests that dysregulated pain modulation may play an important role in FM (Okifuji et al., 1999). Other factors such as elevated levels of substance P (SP) in cerebrospinal fluid, a mediator of nociception (Schwarz et al., 1999), altered serotonin metabolism (Offenbaecher et al., 1999) and lower melatonin secretion during sleep (Press et al., 1998) have been reported.

Cytokines are essential messenger molecules in the regulation of immunological activity. There are hundreds of cytokines and their network of activity is very complicated. It has become clear that cytokine networks can be perturbed at different levels and have very significant and strong effect on the overall autoimmune process (Roitt et al., 2001).

There are some hypotheses and pilot studies (Wallace et al., 1989 and Hader et al., 1991) suggesting that cytokines may play a role in FM. However, their presence and functions have not yet been characterized (Gur et al., 2002a).

Because brain cells express cytokine receptors and lymphocytes express opiate receptors and bind substance P, peptide neurotransmitter in primary afferent neurons of unknown source in CSF, so there may be a neuroimmune – cytokine link relevant to FM (Hader et al., 1991).

The hypothalamic pituitary axis (HPA) and sympathetic nervous system (SNS) are linked to cytokines and T-lymphocytes. Catecholamine and neurokinin K promote the release of IL-1, IL-6 and TNF-α which activate the HPA axis (Pillemer et al., 1997).

Moreover, The SNS innervates lymphocytes especially T cells. Substance P, whose spinal fluid levels are increased in FM, stimulates the release of IL-6. IL-6 can activate the SNS (Papanicolaou et al., 1998).

IL-6 produces fatigue and pain in healthy people, decreases cognitive function, correlates with depression, influences the hyperalgesia of corticosteroid withdrawal and promotes B and T cell proliferation (Oka et al., 1995).
It was also found that substance P, induced IL-8 expression which can be blocked by IL-1 receptor antagonist (IL-1Ra). IL-8 is a proinflammatory chemokine that induces neutrophil trafficking across the vascular wall and is a mediator of sympathetic pain. (Cunha et al., 1991). On the other hand, IL-10 can block pain, increase B-cell levels and promote energy by down-regulating type 1 responses and decreases IL-6 and TNF-α production by monocytes (Maes et al., 1998).

Aim Of Work:

The aim of this study was to evaluate the possible role of cytokines in FM, by measuring IL-6, IL-8, and IL-10 serum levels in FM patients and correlating these levels with the clinical manifestations of the patients.

PATIENTS AND METHODS

Patients:

Twenty female fibromyalgia (FM) patients were selected from the Out-patient Clinic of the Rheumatology & Rehabilitation Department of Ain Shams University Hospitals. Ten age-matched apparently healthy control females were included in the study over a period of six months. Patients fulfilled the American College of Rheumatology (ACR) criteria for FM (Wolfe et al., 1990).

Exclusion criteria (Gur et al., 2002a) were:

- Recent or past history of psychiatric disorders.
- Immuno-compromized subjects.
- Subjects with neurological, inflammatory, endocrine, or clinical significant chronic disease, such as diabetes mellitus, rheumatoid arthritis, inflammatory bowel disease, and organic brain disorders.
- Subjects with abnormal liver function tests.
- 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.

Methods:

All patients and controls were subjected to the following:

- Full history taking.
- Pain intensity and sleep quality were assessed with a numeric rating scale (NRS) using a scale from 0 to 10 where 0 corresponded to the
lowest and 10 to the highest sleep quality and pain intensity (Roizenblatt et al., 2001).

- Fatigue was measured according to the fatigue severity scale (FSS). The FSS is a 9 item questionnaire where each item is scored from 1 to 7 and the FSS score is the mean of the 9 statement score (Krupp et al., 1989).

- Depression and anxiety were assessed with the Beck depression and Beck anxiety scales. The Beck depression inventory is a well known 21 item scale that measures mood and behavior characteristic of depression (Beck et al; 1979). The Beck anxiety inventory is a 21 item instrument used to measure the severity of anxiety while discriminating anxiety from depression. Scores range from 0 to 63 (Gorenstein and Andrade, 1996)

- Thorough clinical examination with special attention to the number of tender points, and exclusion of major clinical conditions other than FM.

- C-X-ray for both hands, feet, chest, and sacroiliac joints: all subjects had normal findings.

**Laboratory Tests:**

Venous blood was collected from each person into a sterile tube and allowed to clot at room temperature for 30 minutes. Serum was aspirated, stored at -20°C until the time of use. The following laboratory investigations were done for each subject:

- Complete hemogram using the coulter T660 cell counter (Hemoglobin level, total and differential leukocyte count).
- Erythrocyte sedimentation rate (ESR) estimation with Westergren method.
- Fasting and post prandial blood sugar, liver function tests (SGPT, SGOT), and kidney function tests (urea & creatinine).
- Thyroid function tests: Quantitative determination of total triiodothyronine “T3”, thyroxin “T4” & thyrotropin “TSH” with electro-chemiluminescence immunoassay (Roche Diagnostics, USA, supplied by BM Egypt).
- Antinuclear antibody detection in the serum with indirect immunofluorescence, using Quantafluor Universal FITC conjugate Kallestrad Kit (USA) supplied by Alkan, following the manufacturer’s instructions.
- Rheumatoid factor detection with latex agglutination test kit (Biosystems, Spain) supplied by Nature.
Estimation of IL-6, IL-8, and IL-10 serum levels using commercially available enzyme linked immunosorbent assay (ELISA) kits (CYT ELISA, CYT IMMUNE Science, Inc., Maryland, USA) purchased from clinilab. The assay was conducted according to the manufacturer’s instructions. Absorbance was read at 450 nm; the interleukin concentration in samples was obtained by extrapolation from a standard curve, and expressed in pg/ml.

Statistical Analysis:

Results were expressed as mean ± standard deviation. The student’s t-test was used to calculate significance. The Spearman’s row rank correlation coefficient “r” test was used for finding correlation between variables. p value less than 0.05 was accepted as statistically significant.

RESULTS

The patients' age ranged from 23 – 40 years (mean 27.25±4.09); with disease duration range from 6 – 30 months (mean 18.19±6.6). Controls age ranged from 23 – 39 years (mean 27.42±4.25). The clinical characteristics of patients are summarized in table (1).

Table (1): Clinical Characteristics of Patients with fibromyalgia:

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>MEAN ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tender points</td>
<td>14.81 (± 1.91)</td>
</tr>
<tr>
<td>NRS pain score</td>
<td>6.70 (± 0.89)</td>
</tr>
<tr>
<td>NRS sleep score</td>
<td>3.7 (± 1.9)</td>
</tr>
<tr>
<td>FSS fatigue score</td>
<td>4.9 (± 1.5)</td>
</tr>
<tr>
<td>Beck depression score</td>
<td>12.4 (± 8.7)</td>
</tr>
<tr>
<td>Beck anxiety score</td>
<td>16.5 (± 7.8)</td>
</tr>
</tbody>
</table>

NRS = Numeric Rating Scale  FSS = Fatigue Severity Scale

Table (2) shows that there was a significant (p < 0.05) increase of IL-6 and IL-8 serum levels in FM patients compared to controls. On the other hand, there was no statistically significant (p > 0.05) difference between patients and controls regarding IL-10 serum levels.

Table (2): Measurements of interleukin – 6 (IL-6), IL-8, and IL-10 serum levels in patients with fibromyalgia and healthy controls.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>p Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>p &gt; 0.05</td>
<td>= Non significant difference</td>
<td>p &lt; 0.05   = Significant difference</td>
</tr>
</tbody>
</table>
Table (3) illustrates that in FM patients, IL-6 serum levels showed a positive significant correlation ($r = +0.62$) with fatigue severity score, a negative significant correlation ($r = -0.59$) with sleep score; IL-8 serum levels showed a positive significant correlation ($r = +0.68$) with number of tender points, and a positive significant correlation ($r = +0.54$) with pain score; IL-10 showed no significant correlation with any clinical variable.

Table (3): Correlation between serum IL–6, IL–8, IL–10 and clinical variables in FM patients.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>IL – 6(Pg/ml)</th>
<th>IL – 8(Pg/ml)</th>
<th>IL – 10(Pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tender points</td>
<td>0.31</td>
<td>0.68</td>
<td>0.27</td>
</tr>
<tr>
<td>NRS pain score</td>
<td>0.28</td>
<td>0.54</td>
<td>0.20</td>
</tr>
<tr>
<td>NRS sleep score</td>
<td>-0.59</td>
<td>0.36</td>
<td>0.30</td>
</tr>
<tr>
<td>FSS fatigue score</td>
<td>0.62</td>
<td>0.25</td>
<td>0.29</td>
</tr>
<tr>
<td>Beck depression score</td>
<td>0.25</td>
<td>0.31</td>
<td>0.28</td>
</tr>
<tr>
<td>Beck anxiety score</td>
<td>0.29</td>
<td>0.28</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* = Significant Value
NRS = Numeric Rating Scale
FSS = Fatigue Severity Scale

DISCUSSION

After almost 2 decades of interest, FM disorder remains an enigma, with little knowledge of cause, treatment, or outcome (Fitzcharles et al., 2003). Evidence linking FM disorders (disturbed sleep, hyperalgesia, cognitive dysfunction, fatigue, stress and anxiety) to cytokines (Vogontzas et al., 1997 & Vanderrhaeghe 2001), led us to evaluate IL-6, IL-8, and IL-10 serum levels in FM patients, correlating these levels with the clinical manifestations of the patients.

IL-6 is a multifunctional cytokine primarily responsible for induction of hepatic synthesis of acute phase proteins, as well as enhancement of immunoglobulins production by B-lymphocytes that characterize many chronic human autoimmune and inflammatory diseases (Heimrich et al., 1990); and is considered to be a major marker of systemic response to the inflammatory process (Kishimoto, 1989).

In the present study, FM patients showed significant elevated IL-6 serum levels as compared to controls, a finding may be due to a unique inflammatory response responsible for the characteristic pathophysiological and clinical manifestations in FM. In accordance with our findings, Vanderhaeghe, (1999); and Wallace et al. (2001), suggested that IL-6 plays an etiopathogenetic role in FM.
Moreover, Salemi et al. (2003) showed the presence of IL–6 in the skin of certain FM patients within fibroblast like cells and mononuclear cells at sites of nerve tissue. They suggested an inflammatory component in the skin of certain FM patients as neurogenic inflammation may occur when substance P and other neuropeptides released from sensory nerve fibers produce an inflammatory response.

Clinical studies showed that more than 75% of FM patients complain of poor sleep (light, unrefreshing, accompanied by generalized stiffness and / or aching, and profound fatigue upon awakening (Buchwald et al., 1994). In the present study, elevated IL-6 serum level in FM patients showed a negative significant correlation with sleep score (i.e. patients who have sleep disturbances), and a positive significant correlation with fatigue score, a finding in agreement with those of Vgontzas et al. (1997); and Spath – Schwalbe et al. (1998), which provides further evidence for the role of IL-6 in the pathology and etiology of FM, especially sleep disturbances and fatigue.

The diurnal sleep – wake rhythm is the result of oscillatory mechanisms that involve brain IL-1 and the neurohormones of the hypothalamic pituitary axis (Krueger & Obal, 1993), whereas IL-1 is believed to have a central role in the induction of slow wave sleep “SWS” (Roitt et al., 2001). Disrupting SWS is probably an important factor in the pathophysiology of FM (Lentz et al., 1999).

The association between elevated IL-6 serum levels and sleep disturbances in FM patients in this work might be explained by the fact that the immune system is integrated with the nervous and endocrine systems, whereas, IL-1, / TNF-α and IL-6 have direct effects on the hypothalamus and pituitary (Roitt et al., 2001). Thus, it might be reasonable to assume that there would have been a possible contribution of elevated IL-6 serum levels on the interruption of slow wave sleep induction by IL-1, and consequently disturbed sleep.

Fatigue, severe fatigue lasting 24 hours after minimal activity, is one of the most common major symptoms reported by FM cases (White et al., 1999). Fatigue is a disabling phenomenon and may cause longstanding sick leave or other disability problem related to work and daily life in FM patients. IL-6 in particular is thought to worsen the symptoms of autoimmune disease and FM immediate disappearance of fatigue following the administration of anti IL-6 receptor antibody was observed by Nishimoto et al. (2000). This could be mediated through disruption of the blood brain barrier or other mechanisms since it is known that peripherally derived
cytokines may communicate with the brain (Laye et al., 1999). Therefore, blockade of IL-6 signaling, and IL-6 overproduction, with a humanized anti-IL-6 receptor antibody might be a novel, promising and effective therapy in FM patients.

Fibromyalgia (FM) is felt to be a syndrome characterized by abnormal central sensory processing of pain signals and is thought to arise from a combination of interactions between neurotransmitters, external stressors, behavioral constructs, hormones and the sympathetic nervous system. Although cytokines are suspected to play a role in FM, their precise dynamics has escaped elucidation (Wallace et al., 2001).

It was suggested that IL-8 may play an important role in the occurrence of pain in FM (Gur et al., 2002a). IL-8 is a member of a family of proinflammatory cytokines that have been referred to as chemokines. It is a potent neutrophil chemotactic and activating factor, and presumably plays a major role in neutrophil focal recruitment at inflamed sites (Al-Dahan et al., 1995).

In this study, serum IL-8 levels were significantly elevated in FM patients as compared to controls, a finding that implies the involvement of IL-8 mediated inflammatory – response in the pathogenesis of FM. Widespread persistent musculoskeletal pain is the cardinal symptom of FM. The distinctive sign is tenderness on pressure at specific sites over the muscles or muscle attachments (Croft et al., 1994).

We found that the elevated IL-8 serum levels were related to pain intensity and number of tender points. This indicates that IL-8 is a promoter of sympathetic pain and may play an important role in FM, as reported by Gur et al. (2002a) & Gur et al. (2002b). In consistence with our findings, Wallace et al. (2001) suggested that the increased central repetitive sensitization of sensory inputs via afferent C fibers and autonomically derived B fibers into the dorsal root ganglia associated with FM initially leads to a greater proliferative response in IL-8 factor that mediates sympathetic pain.

Because the current theory regarding the pathogenesis of FM is focused towards central dysregulation of pain processing mechanisms (Fitzcharles et al., 2003), some treatment interventions with anti-IL-8 receptor antibody might facilitate the shift of pain threshold toward mortality in FM patients.

IL-10 is an anti inflammatory cytokine produced by Treg or Tr1 cells (CD4+ T cell subset able to regulate immune response), in addition to TH2 cells. It down regulates B7 and IL-12 expression by antigen presenting cells “APCs”, which in turn inhibits TH1 activation. It effectively inhibits
the production of pro-inflammatory cytokines as IL-6, IL-1, and TNF-α (Roitt et al., 2001). It also exerts its regulatory function by enhancing the production of anti-inflammatory IL-1 receptor antagonist, which has been shown to dampen the inflammatory activity (Casatell et al., 1994).

Concerning the IL-10 serum level in the present study, FM patients showed a slight reduction as compared to controls, but this reduction did not achieve the level of statistical significance, a finding in agreement with that of Wallace et al. (2001). Because cytokines are extremely potent, acting at picomolar (10^{-12}) and sometimes even femtomolar (10^{-15}) levels (Roitt et al., 2001), we can speculate that the slight lowering in IL-10 serum levels in FM patients might provide an interesting insight in the pathogenesis of FM, whereas the enhanced inflammatory activity (manifested by elevated IL-6 and IL-8 serum levels) observed in FM patients in the present study might be assumed to be due to insufficient suppression of the deleterious effects of those proinflammatory cytokines.

It has not yet been determined however, whether elevated cytokines are the direct cause of FM or merely secondary to another factor. Factors that could contribute to cytokines increase and be a more direct cause of the ailment are the other immune problems, abnormal hormone activity, or sleep disturbances (Addington, 2002).

While cytokine related drug therapies are starting to have some benefit for conditions like rheumatoid arthritis, that is not the case yet for FM. Dr. Patarca (2001) has extensively studied the topic and he says medications are not available “mainly because nobody knows which cytokine system in particular to target and because of the complexity of the cytokine network components.” But research on the topic continues and a number of cytokine altering medications are currently being developed, so it may be that in the not too distant future, medications to regulate cytokine difficulties for FM may exist.

Conclusion:

The data of this study provide further evidence for the crucial, critical, and important role of cytokines in the pathogenesis of FM. IL-6 may be related to the occurrence of sleep disturbances and fatigue. Further studies are needed to clarify whether a possible association between elevated IL-6 and perturbation of IL-1 induction of sleep might exist. IL-8 may be related to pain intensity and number of tender points. Strategies to decrease the level of cytokines, as humanized anti-IL-6 receptor antibody (Nishimoto et al., 2000) may constitute novel hopes for immunoregulatory treatment of FM patients.
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