The Role of Interleukin-8 in Patients with Primary Fibromyalgia

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ABSTRACT:

BACKGROUND:

Fibromyalgia syndrome (FMS) is a common rheumatologic syndrome with multiple manifestations and associated with many diseases, it characterized by chronic wide spread muscular pain and tenderness. Further, circulatory levels of pro-inflammatory cytokines like IL-8 and others may be altered in FMS patients, possibly associated with their symptoms.The objective of the study is to measure IL-8 concentration and to evaluate its role in the pathogenesis of primary FMS.

OBJECTIVE:

Fifty patients with primary FMS were included in the study (37 females and 13 males) the age range of (17-65) years (Mean ± SD) (40.13 ± 12.0) years, and thirty healthy individuals volunteers (21 females and 9 males), whose age and sex matching with FMS patients, age ranging (18 - 63) years, (Mean ± SD) (36.1 ±10.0) years.

METHODS:

IL-8 concentration was measured in sera of patients and controls by ELISA kit. Anthropometric measurements like body mass index (BMI) and waist circumference (WCr) were taken ,besides other features like sleep disturbance , emotional distress , and fatigue were reported.

RESULTS:

IL-8 concentration was higher in FMS patients than controls(40.24±22.0) pg/ml vs. (19.16±7.8) pg/ml. This elevation was highly significant statistically (p=0.000).Other measurements in patients group like BMI, WCr were(28.39±5.0) Kg/m^2 and (100.34±13.21) cm respectively .These values were highly significant when compared to their control group (p=0.002)and (p=0.008) respectively .Clinical features like sleep disturbance, emotional distress ,and fatigue showed highly significant difference between the two groups . No significant differences were reported with respect to age and sex.

CONCLUSION:

The result of the current study suggest that interleukin-8, (IL-8) might have a role in the pathogenesis of FMS.

KEYWORDS: interleukin-8, fibromyalgia.

INTRODUCTION:

According to the criteria of the American College of Rheumatology in 1990 (ACR); fibromyalgia (FMS) is characterized by chronic widespread pain in all 4 quadrants of the body at least 3 months duration associated with tender points and associated with constitutional symptoms of fatigue, aching, and nonrestorative sleep[1]. The etiology of FMS remains elusive, neurochemicals may play a key role in FMS, in several controlled studies, spinal fluid levels of nerve growth factor and substance P were elevated [2] and spinal fluid levels of serotonin products are decreased in FMS [3] .

Interleukin-8 (IL-8) is a small protein [4] , a member of CXC chemokines family (chemotactic cytokines) , which are a small inducible proteins (6-15 kDa) characterized by their capacity to attract subsets of leukocytes , more than 20 chemokines have been isolated in humans [5].They were divided into four classes, according to numbers and spacing of cysteines in their sequence : CXC , CC , C , and CX3C where C is cysteine and X is any amino acid residue [6] . IL-8 is produced by macrophages, and epithelial cells

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which store IL-8 in their storage vesicles (the Weibel – Palade bodies), and any cells with Toll-like receptors (TLRs) [7].Although FMS is non-inflammatory process, there are a lot of studies which demonstrated that cytokines have a place among proposed FM paradigms since 1988.However there are contradictory results in the literature.In Sperber *et al* study [8] , serum levels of interleukin-1(IL-1),(IL-2), (IL-6) among FMS patients did not differ from healthy controls. Gür *et al* [9] , showed a significant elevated serum IL-8 and IL-2r but not IL-1 or IL-6.Wallace *et al* [10] postulated increased serum IL-1R and IL-8 but not IL-1β,IL-2, IL-10, soluble IL-2 Receptors(sIL-2R),interferon (INF-γ) in FMS. Űceyler *et al* demonstrated in contrasts that pro-inflammatory cytokines IL-2, IL-8, TNF-α and transforming growth factor-β1 did not increase but anti-inflammatory cytokines IL-4 and IL-10 were lower in FMS patients than in controls [11] .In the present study, a trail was made to investigate the role of IL-8 in patients with primary FMS and its association with the pathogenesis of the syndrome and if there is association between circulating IL-8 and pain intensity, body mass index , waist circumference , age and gender in FMS.

SUBJECTS AND METHODS:

SUBJECTS:

The study has included 50 patients with primary FMS (37 females and 13 males) the age range of (17-65) years (Mean ± SD) (40.13 ± 12.0) years. The clinical diagnosis of these patients was confirmed by Consultant Rheumatologists of the Baghdad Teaching Hospital according to the ACR 1990 criteria for the diagnosis of FMS. Patients with primary fibromyalgia were included in this study. Thirty healthy individuals volunteers (21 females and 9 males), whose age and sex matching with FMS patients, age ranging (18 - 63) years, (Mean ± SD) (36.1 ±10.0) years. They had no musculoskeletal complaints or lower back pain and did not seek any medical help for pain. A pre-tested questionnaire was designed to obtain information from both patients and control group about past medical and drug history .

Inclusion criteria:- Known cases of FMS approved by clinical, laboratory,and radiological diagnosis. Patients on medical treatments that never affect the laboratory tests.

Exclusion Criteria: Diabetes mellitus (DM) ,Rheumatoid arthritis (RA),Systemic lupus erythematosis (SLE), Sjögren's syndrome (SS), Osteoarthritis (OA),Sleep apnea ,Patients on steroid therapy, Chronic renal failure, Chronic liver disease, Previous breast surgery, Inflammatory systemic disease or infection, Serious cardiopulmonary, vascular or other internal medical condition. Medication that may influence the level of cytokines (e.g. local corticosteroids , biological agents).

Blood collection:

After overnight fasting venous blood samples (10 ml) were aspirated from each patient and control at 9.00 am - 12.00 pm using disposable plastic syringes . Seven ml of the blood samples were allowed to clot in plane tubes at room temperature for (20-30) minutes. Sera were separated by centrifugation at 3000 rpm for 10 minutes. For each sample, the serum was transferred into plastic plane tubes and kept frozen at (-20C̊) until the time of assay. The rest 3 ml blood samples were collected in EDTA tubes for ESR, PCV, and WBC tested by routine work to exclude inflammatory reasons.

METHODS:

Serum Interleukin-8 was determined by immunotech IL-8 ELISA kit, which is solid phase enzyme- linked immunosorbent assay based on the sandwich principle .Kit used was from Immunotech- France. Pain was measured using Visual Analogue Scale (VAS).

Statistical analysis: To compare the significant of the difference in the means values at any two patients and controls, SPSS (social process statistical system) was used. Student t-test was applied ($P < 0.05$) was considered statistically significant , and the correlation coefficient (r) test is used to describe the association between different parameters studied .

RESULTS:

As shown in table-1 , there was significantly high concentration of IL-8 in patients with FMS than healthy controls(40.24±22.0) pg/ml vs. (19.16±7.8) pg/ml, (p=0.000). Patients and controls were age, sex match (p=0.124),(p=0.698) respectively .There was a statistically significant difference in BMI and WCr between patients and controls(28.39±5.0) Kg/m^2 and (100.34±13.21) cm ,(p=0.002),(p=0.008) respectively.

Table 1: Shows the mean values of all parameters measured for patients and control group, (Mean ± SD) values for Age, BMI, WCr, IL-8, in FMS group (n=50) and control group (n=30)

Characteristic	FMS patients n=50 Mean ± SD	HC n =30 Mean ± SD	P-value	Sig
IL-8 (pg/ml)	40.24 ± 22.0	19.16 ± 7.83	0.000	HS
BMI (Kg/m^2)	28.39 ± 5.0	25.14 ± 3.19	0.002	HS
WCr (cm)	100.34 ± 13.21	92.63 ± 10.76	0.008	HS
Age (year)	40.18 ± 12.08	36.10 ± 10.02	0.124	NS

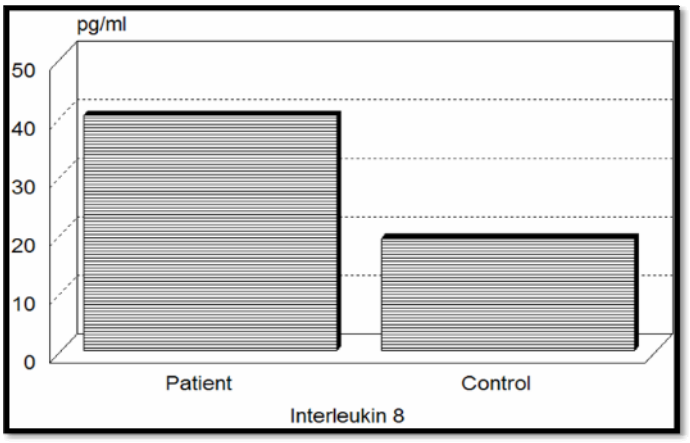

Figure 1 :Mean values for serum IL-8 level in FMS patients and control group.

Table 2 :Non- Clinical feature of the study

Characteristic	FMS (n=50) n (%)	HC (n=30) n (%)	Total (n=80) n (%0)	P-value	Sig
Gender Female Male	 37 (74.0 %) 13 (26.0 %)	 21 (70.0 %) 9 (30.0 %)	 58 (72.5 %) 22 (27.5 %)	0.698	NS
Age (year) <20 20-40 41-60 61-80	 2 (4.0 %) 26 (52.0 %) 19 (38.0 %) 3 (6.0 %)	 1 (3.3 %) 22 (73.3 %) 6 (20 %) 1 (3.3 %)	 3 (3.8 %) 48 (60.0 %) 25 (31.3 %) 4 (5.0 %)	0.30	NS
BMI (Kg/m$^{2)}$ Lean (<18.5 kg/m2) Normal (18.5-24.9kg/m2) Over (25-29.9 kg/m2) Obese (≥ 30 kg/m2)	 1 (2.0 %) 11 (22 .0%) 20 (40.0 %) 18 (36.0 %)	 — 15 (50.0 %) 12 (40.0 %) 3 (10.0 %)	 1 (1.25 %) 26 (32.5 %) 32 (40.0 %) 21(26.25 %)	0.002	HS

Table 3 :Clinical features of the study

Characteristic	FMS (n=50) n(%)	HC (n=30) n(%)	Total (n=80) n(%0)	P-value	Sig
Sleep disturbance +ve -ve	 47 (94.0 %) 3 (6.0 %)	 3 (10.0%) 27 (90.0 %)	 50 (62.0%) 30 (38.0 %)	0.000	HS
Emotional distress +ve -ve	 50 (100.0 %) —	 6 (20.0 %) 24 (80.0 %)	 56 (69.7 %) 24 (30.3 %)	0.000	HS
Fatigue -ve Mild Moderate Severe	 — 11 (22.4 %) 25 (51.0 %) 14 (27 .0 %)	 22 (73.3 %) 8 (26.7 %) 0 (0.0 %) 0 (0.0 %)	 22 (27.8 %) 19 (24.1 %) 25 (31.6 %) 14 (16.5 %)	0.000	HS

DISCUSSION:

In this study, serum IL-8 concentration was significantly higher in patients with FMS than in healthy controls table -1 and figure -1.The increase in IL-8 was independent of body mass index and waist circumference and did not correlate with the number of tender points, pain intensity measured by VAS or any other biochemical parameter measured in the study. The variation in cytokine level in this study might reflect the activation of specific intracellular mechanisms, as consequence of biochemical and functional alterations in FMS patients [(12)]. IL-8 was up regulated by pro-inflammatory signals such as IL-1, TNF-α, IL-17 [(13)] and inhibited by anti-inflammatory cytokines IL-4, IL-10, and IFN-γ. Pro-inflammatory cytokines can be useful markers in the study of chronic pain because they are known to enhance nociception by changing ion channels regulated through second messenger cascades [(14)]. Interleukins are known to contribute to cyclooxygenase-2(COX-2) and mediate release of prostaglandins resulting in increased voltage-dependent calcium inflow in nociceptive fibers. Activity and metabolism of sensory fibers are mediated by interaction with inflammatory infiltration produced by immune cells in response to tissue injury [(15)]. There are multiple theories regarding the pathophysiology of FMS,one leading hypothesis suggests that FMS may involve aberrations of the hypothalamic –pituitary-adrenal axis that are associated with or caused by cytokines imbalance, where cytokines affect the functions of the CNS through autonomic neuroendocrine and behavioral mechanisms ,noting that IL-8 promotes sympathetic pain and IL-6 induces hyperalgesia, fatigue and depression [(16)].Mast cells have been proposed as a target for the increased level of both corticotrophin releasing hormone (CRH) and Substance P outside the brain leading to the enhancement of the inflammatory process that could contribute to pain. Studies show that human mast cells express functional CRH receptors and CRH can induce selective release of vascular endothelial growth factor that could enhance inflammation [(17)], once inflammation occurs; IL-1 could then stimulate mast cells to release IL-6 selectively. In other clinical hypothesis , fascia has been shown to be able to have contractile force *in vitro* and this fascial contractility is thought to contribute to the incredible feats of strength humans can perform emergencies –situations in which the sympathetic nervous system is also dominant [(18)], so in response to chronic excess fascial tension, fibroblasts would likely overproduce collagen and extracellular matrix in continuous attempt to respond to the increased mechanical stress. However due to inadequate growth hormone stimulation of fibroblast, there may be an impaired fascial healing and unable to repair muscle microtrauma leading to fibroblasts over production of collagen and secreting cytokines resulting in chronic fascial inflammation and prolonged irritation of fascial nociceptors leading to central sensitization [(19)].

Although there is theoretical support for the view that cytokines may be associated with the pathogenesis and core symptoms of FMS, an integrated review published in 2010 screening all clinical articles and researches involved in cytokines and its role in FMS found a limited empirical evidence to support these relationships .Although the use of cytokines as a biomarker must

be considered exploratory at this time due to lack of consistent empirical finding , biobehavioral research focused on understanding the relationship of FMS with cytokines which may lead to better understanding of this complex syndrome[20].

CONCLUSION:

In conclusion our findings suggest the possibility of underlying inflammatory process in FMS. Other inflammatory markers need to be tested like TNF-α, IL-1,IL-1Ra, IL-10, and IL-4, in sera of primary Fibromyalgia patients to evaluate their role in the development of primary FMS can be recommended for further investigations besides, detection of the expression of IL-8 and other inflammatory markers in the peripheral blood mononuclear cells, muscle biopsies, and muscle biopsies of tender points (if possible) in patients with primary FMS. This should add more information on the role of these cytokines in the pathogenesis of fibromyalgia.

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