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The CorrelationStudy between Serum Concentrations of pro-inflammatory cytokines and acute phase markers in Rheumatoid Arthritis Patients in AL-NajafGovernorate

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ABSTRACT

Rheumatoid arthritis is a common autoimmune disease of the musculoskeletal system that is associated with considerable morbidity and diminished quality of life. Serum samples were obtained from 70 patients with RA who had visited the Division of Rheumatology at AL-Sadder Teaching Hospital in AL- AL-Najaf City and 25 age- and sex-matched healthy controls, and the clinical parameters of disease were assessed, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor (RF).Serum concentrations of IL-6, (TNF-α), (IL)-1α, (IL)-8 and serum level of ferritin were measured using an enzyme-linked immunosorbent assay (ELISA). Serum concentrations of IL-6, (TNF-α), (IL)-1α, and (IL)-8weresignificantly elevated ($P<0.0001$) in patients with RA compared to those of healthy controls. Serum levels of ferritin were also significantly elevated ($P<0.0001$) in patients with RA compared to those of healthy controls. There were a good significant correlation between these cytokines and acute phase markers. It has been concluded that the serum concentrations of IL-6, (TNF-α), (IL)-1α, and (IL)-8 were significantly elevated in patients with RA and strongly correlated with acute phase markers. These findings suggest a possible role for these cytokinesin the pathogenesis of RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disease that causes inflammation mainly in the synovium and produces destruction and deformity of the joints. The etiology of RA remains unclear, but it is known to be associated with genetic and environmental factors.[1]

Various proinflammatory cytokines, such as IL-6, tumor necrotic factor (TNF)-α, interleukin (IL)-1α, and IL-8, are increased in the synovial tissue or synovial fluid of patients with RA.[2,3]Increased levels of proinflammatory cytokines lead to the proliferation of synovial tissue, and thereby cause damage in the articular cartilage and bone destruction in the adjacent area.[4,5]In particular, IL-6 is a cytokine with various functions. When IL-6 is activated, acute inflammatory responses such as fever or anemia are induced. IL-6 promotes the proliferation of B cells and thus is involved in the production of the rheumatoid factor. [6,7]Tumor Necrosis Factor (TNF) is a multifunctional cytokine with potent proinflammatory effects, and is implicated

in many inflammatory and autoimmune diseases and is a member of a group of cytokines that stimulate the acute phase reaction [8].

Proinflammatory cytokines such as IL-1 and TNFα and chemokines are key mediators of the inflammation attracting inflammatory cells to the synovium which induces bone and joint destruction in RA.[9, 10]

The proinflammatory cytokines IL-1, TNFα, and IL-6 in particular, were responsible for the induction of several plasma proteins such as C-reactive protein (CRP) and ferritin synthesis in a hepatic cell line.[11,12]

The current study was sought to examine whether serum levels of IL-6 TNFα,IL-1, and IL-8 areincreased in patients with RA and whether the increased levels are significantly correlated with acute phase markers. The serum concentrations of these cytokines were compared in patientswith RA and those in normal controls and then investigated the correlation between serum levels of proinflammatory cytokines and the acute phase markers alterationsof RA.

MATERIALS AND METHODS

Subjects and clinical assessment

This study was conducted in 70 patients who had visited the Division of Rheumatology at AL-Sadder Teaching Hospital in AL-Najaf city between February and August 2011, and who fulfilled the American College of Rheumatology (ACR) 2010 revised criteria for the diagnosis of RA.[13]Twenty fiveage-and sex-matched healthy adults without any evidence of chronic inflammatory diseaseserved as the controls. The patients underwent thorough clinical and laboratory evaluation, including complete medical history, seropositivity test for rheumatoid factor (RF), C-reactive protein (CRP), and estimation of erythrocyte sedimentation rate (ESR).

Study design

The group of patients enrolled in the natural history protocol was classified according to the duration of RA disease [14] as follows:

1. Group I (GI): the group of patients with disease length of (less than one year) was considered as the group with very early disease duration.
2. Group II (GII): the group of patients with disease length of (1-5) years was considered as the group with early disease duration.
3. Group III (GIII): the group of patients with disease length of (5-15) years was considered as the group with median disease duration.
4. Group IV (GIV): the group of patients with disease length of (15-25) years was considered as the group with long disease duration.
5. Group V (GV): the group of patients with disease length of (more than 25) years was considered as the group with very long disease duration.

Methods

1. Erythrocyte sedimentation Rate (ESR)

The International Committee on Standardization in Hematology (ICSH) recommends the use of the Westergren method[15,16].

2. C - reactive protein (CRP) serology test

The CRP-latex test kit was used according to the manufacturing company instructions (SPINREACT, S.A. Spain).

3. Rheumatoid Factor (RF) Latex serology test

Rheumatoid factor (RF) Latex serology test kit was used according to the manufacturing company instructions (LTA s.r.l. Italy).

4. Measurement of IL-6, TNFα, IL-1α, IL-8 and serum ferritin levels

The serum concentration of IL-6 was measured using an AssayMaxenzyme-linked immunosorbent assay (ELISA) kit according to the manufacturing company (Assay pro, USA).

Statistical analysis

Data analyses were performed with GraphPad Prism version 5.04 Software (GraphPad Software Inc., San Diego, CA, USA). All of the descriptive variables were expressed as the mean ± standard error (SE). The correlations between the concentrations of IL-6and acute phase markerswere tested using Pearson's correlation test. The group analyses were performed using one-way ANOVA and Tukey's post-hoc analyses.For all tests, a *p* value less than 0.05 was considered statistically significant.[17]

RESULTS

Clinical characteristics of the study subjects

The demographic and clinical data of the subjects are shown in Table 1. The mean age of the 70 patients with RA was 47.9 ± 3.32 years (range: 27-75 years), and the patient group was comprised of 13 males and 57 females. The mean disease duration from symptom onset was 11.52 ± .085 years (range: 0.25-31 years). Patients were divided into five groups according to the disease duration: 10 in the very early duration 0.43±0.06 year (range: 0.25-0.58 year), 19 in the early duration 2.28±0.22 (range: 1-4 years), 20 in the median duration 7.8±1.8 (range: 5-14years), 11 in the long duration 19.4±0.57 (range: 18-21 years), and 10 in the very long duration 27.7±1.6 (range: 25-31 years).The mean age of the healthy controls was 42±2.03 years (range: 24-75 years), and the control group was comprised of 6 males and 19 females.

Table 1: Clinical characteristics of the study subjects.

Character		RA patients (n=70)				
	Healthy Control	Very early Duration	Early Duration	Median Duration	Long Duration	Very long Duration
Number of subjects	25	10	19	20	11	10

Age (years) (range)	42±2.03 (24-75)	44±3.2 (31-54)	44±3.1 (27-76)	49±2.95 (34-73)	48±5.1 (37-70)	52±2.28 (49-59)
Female/male ratio	19/6	9/1	14/5	16/4	9/2	9/1
Disease duration (years) (range)	--	0.43±0.06 (0.25-0.58)	2.28±0.22 (1-4)	7.8±1.8 (5-14)	19.4±0.57 (18-21)	27.7±1.6 (25-31)
%RF positive patients	--	90%	94.7%	90%	81%	90%
ESR (mm/hour)	19.9±0.5	59.1±9.3	52.5±4.8	52.1±6.67	64.18±5.6	46.6±4
%CRP positive patients	--	90%	89%	90%	90.9%	80%

Serum concentrations ofcytokines

The statistical analysis revealed a significant increase (P<0.0001) in IL-6, TNFα, IL-1α, and IL-8 with all groups of patients with RA compared to the healthy control group(table 2).

Table2: Cytokines concentrations (pg/mL) in healthy control group and in the groups of patients suffering from rheumatoid arthritis.

Cytokine (pg/mL)	Healthy control (n=25)	RA patients (n=70)					
		Duration of disease groups					
		Group I (n=10)	Group II (n=19)	Group III (n=20)	Group IV (n=11)	Group V (n=10)	P value
IL-6	6.35±0.40	$33.99\pm3.26_{a}^{***}$	$33.14\pm2.56_{a}^{***}$	$31.41\pm1.98_{a}^{***}$	$29.89\pm2.41_{a}^{***}$	$30.78\pm2.76_{a}^{***}$	$<0.0001^{***}$
TNF-α	5.36±0.48	$33.44\pm2.1_{a,b}^{***}$	$33.77\pm3.19_{a,b}^{***}$	$33.6\pm2.22_{a,b}^{***}$	$41.021\pm4.74_{a}^{***}$	$26.91\pm2.12_{b}^{***}$	$<0.0001^{***}$
IL-1α	2.82±0.25	$29.31\pm3.16_{a}^{***}$	$26.31\pm0.82_{a}^{***}$	$28.01\pm0.65_{a}^{***}$	$27.11\pm0.69_{a}^{***}$	$25.18\pm0.43_{a}^{***}$	$<0.0001^{***}$
IL-8	11.61±0.87	$41.51\pm6.30_{a}^{***}$	$40.26\pm3.77_{a}^{***}$	$49.09\pm3.92_{a}^{***}$	$41.10\pm3.23_{a}^{***}$	$35.93\pm3.80_{a}^{***}$	$<0.0001^{***}$

- Data are expressed as means ± standard error (SE).
- The asterisks indicate significant difference compared to control based on Tukey's multiple comparison test.
- The same letters indicate non-significant difference between groups based on Tukey's multiple comparison test**.**
- P value summary ***

Serum concentrations of ferritin

The statistical analysis showed that the serum levels of ferritin were elevated significantly(P= 0.0066) in groups of patients suffering from RA compared to the healthy control group (Figure 1).

Erythrocyte sedimentation rate (ESR)

ESR of RA patient groups evaluated in this study was shown in Figure 2. The results showed significant increases (P<0.0001) in the ESR in the group of patients with RA particularly in the long duration of the disease compared with the healthy control group.

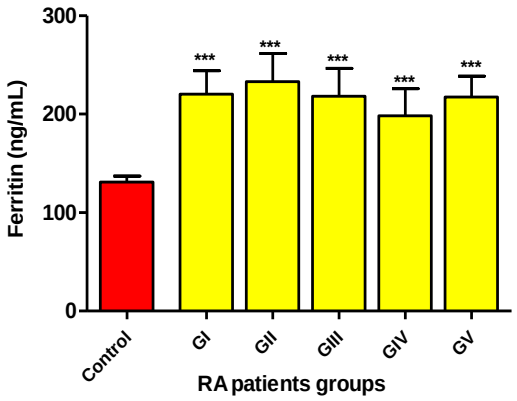

Fig 1: S. ferritin in healthy control group and in the groups of patients suffering from RA

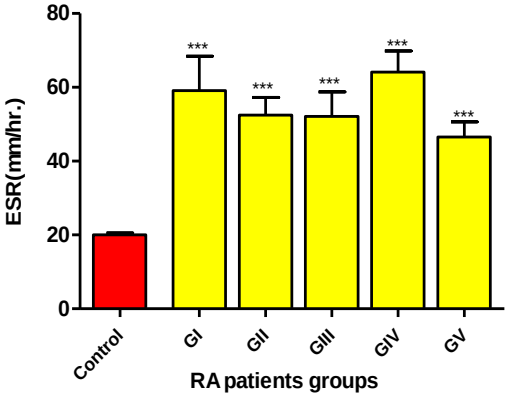

Fig 2: ESR in healthy control group and in the groups of patients suffering from RA

Relationship of cytokines levels to acute phase markers

Serum concentration of IL-6,TNFα, IL-1α, and IL-8 correlated positively and significantly with serum ferritin (r = 0.9480, p<0.0001, r = 0.9539, p<0.0001, r = 0.8758, p<0.0001, and r = 0.9334, p<0.0001, respectively) the highest coefficient correlation being with ESR (r = 0.9776, p<0.0001, r = 0.9752, p<0.0001, r = 0.7719, p<0.0001, and r = 0.9781, p<0.0001, respectively)

DISCUSSION

Table 2showed that serum concentrations of IL-6, TNFα, IL-1α and IL-8were significantly elevated (P<0.0001) in patients with RA compared to those in healthy controls. As seen in previous reports, this finding supports the hypothesis that these cytokinesare involved in the pathogenesis of RA.[18-20]

IL-6 is a cytokine that causes an acute inflammatory response, and it is well-documented that IL-6 plays a crucial role in the pathogenesis of various inflammatory diseases including RA.[21,22]

TNFα plays an essential role in the pathogenesis of rheumatoid arthritis (RA), in view of the fact that anti-TNF treatment is successful in controlling chronic inflammation in RA.[23,24]

Proinflammatory cytokines such as IL-1 and TNFα and chemokines such as IL-8 are key mediators of the inflammation attracting inflammatory cells to the synovium which induces bone and joint destruction in RA.[25,26]

On the other hand, the current study showed that the acute phase markers involved in this study were significantly elevated($P<0.0001$) in patients with RA compared to those in healthy controls (Fig 1 and 2). ESR is a simple and important laboratory test. It is commonly used to assess the inflammatory or acute phase response.[15]In rheumatology; ESR plays an important role in different criteria assessing disease activity and improvement and as a laboratory activity measure in clinical trials.[10] Serum ferritin is an acute phase reactant and elevated serum level of ferritin in RA patients is mainly due to the inflammation.[27,28]Ferritin is the principal iron storage protein participating in iron metabolism. As serum ferritin levels often reflect the amount of storage iron in the body, physicians have measured serum ferritin in order to evaluate iron deficiency or overload. Although a rise in serum ferritin concentration occurs in iron overload, hyperferritinemia (elevation serum levels of ferritin) without it has been reported in some inflammatory diseases and malignancies. Some cytokines have been reported to be responsible for the elevation of ferritin production. However, the hyperferritinemia is not a result, but is profoundly participating in the disease process[29,30].

The proinflammatory cytokines involved in the current study showed strong positive correlation with acute phase markers including ESR and serum ferritin.

Several authors supported our finding in that the different acute phase markers used to assess disease activity in RA showed good correlations with each other and with serum proinflammatory cytokines particularly IL-6 levels. [31-32]

Data from Soo-Jin Chung *et al*., (2011) revealed that the positive correlation was found between serum concentrations of IL-6 and CRP levels at baseline. The proinflammatory cytokines IL-1, TNFα, and IL-6 in particular, were responsible for the induction of several plasma proteins such as C-reactive protein (CRP) and ferritin synthesis in a hepatic cell line[33].

IL-6 is liberated systemically from the site of inflammation and is capable of reaching and acting on hepatocytes, may be the most important cell type due to its systemic actions. The hepatocyte expresses both receptors IL-6R/gp130 and therefore is generally sensitive to the effects of IL-6. The effects on the gene expression are a variable sign, either repressors of gene expression for physiological proteins such as albumin, or gene expression activators for proteins known to be (acute phase reactants) APR: ferritin, CRP, and fibrinogen.[34]

It has been concluded that the serum concentrations of proinflammatory cytokines: IL-6, TNFα, IL-1α and IL-8were significantly increased in patients with RA compared with those of normal controls. The cytokine levels were significantly correlated acute phase markers. These findings suggest that these cytokines might be involved in the pathogenesis of RA and that levels of it might reflect the activity of the disease.

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دراسة العلاقة بين تركيز السايتوكينات في الدمودلائل الالتهاب الحاد لدى مرضى التهاب المفاصل الرثوي في محافظة النجف

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الخلاصة

التهاب المفاصل الرثوي من امراض المناعة الذاتية الشائع والذي يصيب الجهاز العضلي–الهيكلي وهو من الامراض الخطيرة الذي يؤثر على حياة المريض.اجريت الدراسة الحالية على (70) مريض بالتهاب المفاصل الرثوي ارتادوا الى مستشفى الصدر التعليمي في محافظة النجفو(25) شخص طبيعي مقارب لهم بالعمر والجنساعتبروا كمجموعة سيطرة، تم تحديد المعايير السرسرية لكل مريض والتي تضمنت معدل ترسيب كريات الدم الحمراء(ESR) وبروتين عالي التفاعل-C(CRP) والعامل الرثوي (RF) اما تراكيز السايتوكينات ومستوى الفرتين فقد تم قياسها باستعمال جهاز (ELISA).

اظهرت النتائج ارتفاعا معنويا ($P<0.0001$) في تراكيز السايتوكينات بدائية الالتهاب IL-6 و TNFα و IL-1αو IL-8لدى مرضى التهاب المفاصل الرثوي مقارنه بالسيطرة . اظهرت النتائج ايضا حصول ارتفاع معنوي ($P<0.0001$) في مستوى الفرتين لدى مرضى التهاب المفاصل الرثوي مقارنه بالسيطرة. كذلك بينت النتائج وجود علاقة موجبة قوية بين مستوى هذه السايتوكينات ودلائل الالتهاب الحاد المتمثلة بسرعة ترسيب كريات الدم الحمراء (ESR) ومستوى الفرتين في الدم.

نستنتج من نتائج الدراسة الحالية على انالسايتوكينات بدائية الالتهاب IL-6 و TNFα و IL-1α و IL-8قد ارتفعت ارتفاعا معنويا لدى مرضى التهاب المفاصل الرثوي مقارنةبالأشخاص الاصحاء وهذا الارتفاع مرتبط ارتباطا قويا مع التغيرات في دلائل الالتهاب الحاد. وهذا الاستنتاج يدلعلى الدور الذي تلعبه هذه السايتوكيناتفي إمراضيه التهاب المفاصل الرثوي.