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Evaluation of Some Immunological Markers in the Rheumatoid Arthritis Patients

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritic disease associated with remissions and exacerbations and characteristic genetic, clinical, pathological, and immunological features. The present study was designed to evaluate some immunological parameters of some Iraqi patients with RA. The study was carried out on 75 Iraqi RA patients who were referred to the consultantand which divided into 59 female and 16 male, treated and non-treated. The diagnosis of those patients has been performed under supervision of a specialist physician in rheumatology. Enzyme linked immunosorbent assay (ELISA) technique has been applied for the detection of anti-cyclic citrullinated peptide antibodies (anti-CCP) and (Interleukin-1α (IL-1α). The study revealed that the mean age for RA patients was (46.16±1.24) years with age range (20-67) years. The results of (anti-CCP) antibody showed significant increase in non-treated patients 86.67% ($P<0.01$) than Methotrexate (MTX) 70.0%, Etanercept(ETN) 53.33% treatment, also in MTX treated patients was significant increased than in ETN treatment while the mean of (IL-1α) was significant increase in non-treated patients 24.57±3.73 pg/ml than in MTX treated patients 13.54±1.16 pg/ml, ETN treated patients 13.06±0.83 pg/ml and healthy control 13.69±1.61 pg/ml. .

Keywords:,Rheumatoidarthritis,cytokines, ,anti-ccpenzyme linked immunosorbent assay.

تقييم بعض الموشرات المناعية لدى مرضى التهاب المفاصل الرثوي

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الخلاصة

التهاب المفاصل الرثوي (Rheumatoid arthritis) هو مرض مزمن يصيب المفاصل ويمر بأطوار متناوبة من الشفاء والانتكاس حيث ان له صفات جينية، سريرية، مرضية، ومناعية مميزة. صممت الدراسة الحالية لتقييم بعض المعايير المناعية عند المرضى العراقيين المصابين بالتهاب المفاصل الرثوي. اجريت الدراسة على 75 مريض عراقي مصابين بالتهاب المفاصل الرثوي الذين كانوا مشار اليها الاستشاري والتي تنقسم 59 من الاناث و 16 من الذكور المعالجين وغير المعالجين وقد تمتشخيص المرضى من قبل الكادر

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الطبي الاستشاري امراض مفاصل طبق اختبار الانزيم المرتبط الممتز (Enzyme linked Immuno Sorbent Assay) للتحري عن اضداد ببتيد السترولين الحلقي (anti–cyclic citrullinated peptide)، مستوى السايتوكينات(cytokines) (انترلوكينوكين–1 الفا،).اوضحت النتائج ان معدل اعمار مرضى التهاب المفاصل الرثوي كا1.24 ± 46.16وكان المدى العمري للمرضى(67 – 20) سنة.أظهرت نتائج أضداد ببتيد السترولين الحلقي (Anti– ccp) زيادة معنوية محسوسة عند المرضى غير المعالجين86.67% عما هو عند المرضى المعالجين بالميثوتروكسيت (70%) والايتانيرسيبت(53.33%) وكذلك هناك زيادة معنوية عند المرضى المعالجين بالميثوتروكسيت مقارنة مع المرضى المعالجين بالايتانيرسيبت. معدل الانترلوكين –1 (الفا) عند المرضى غير المعالجين 3.73±24.57 وهذا يشكل زياده معنوية محسوسة مقارنة مع المرضى المعالجين بالميثوتروكسيت 1.16±13.54 بيكاغرام / مل وكما هو عند المرضى المعالجين بالايتانيرسيبت (0.83±13.06) وكذلك عما هو عند مجموعة السيطرة 1.61±13.64 بيكاغرام / مل.

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic disease characterized by inflammation and cellular proliferation in the synovial lining of joints that can ultimately result in cartilage and bone destruction [1].. (RA) is an autoimmune disease of unknown cause. The immune system normally protects the body against foreign cells such as a virus or bacteria and can differentiate these from "self" tissues. In RA the synovium, a thin membrane that lines the joints, is seen as foreign and attacked by the immune system causing swelling, tissue damage and pain [2].

Anti-citrullinated protein antibodies (ACPA) are directed against one or more of an individual،s own, post translationally modified proteins, and frequently detected in the blood of RA patients. Anti-CCP assays are the most widely used methods to study ACPA. Anti-CCP have been evaluated in patients with early synovitis, and were found to be more specific than RF for early RA, while having comparable sensitivity [3]. Interestingly, RF and anti-CCP have both been found in blood samples taken several years before disease onset in a subset of patients [4, 5].

Several studies in recent years were conducted for evaluation of inflammatory cytokines such as IL1-α and TNF- α in rheumatologic disorders including rheumatoid arthritis to find new treatment methods base to pathogenesis. These studies reveal disequilirium between stimulatory and inhibitory mechanisms in inflammatory disorders such as rheumatoid arthritis and cytokines (IL1-α and TNF- α) have a leading role in pathogenesis [6,7]. The cytokine level in patients with RA may be a novel approach for treatment of these disease [8]. They described successful treatment of refractory arthritis in patients with RA by infusion of antibodies against tumor necrosis factor – alpha (TNF-α), suggesting a key role for this cytokine in the pathogenesis of chronic arthritis[9].

Methotrexate (MTX) is an anchor drug for the treatment of RA because of its efficacy , acceptable safety, and cost . MTX is used in mono therapy or in combination with either biological agents or other small molecule anti-rheumatic drug [9]. Etanercept (ETN) is a soluble TNFα inhibitor and is efficiently used for the treatment of polyarticular RA [10, 11]. TNFα is a pleiotropic proinflammatory cytokine secreted by different cell types and has effects on both innate and adaptive immune cells [12].

Materials and methods

The study was carried out on 75 Iraqi RA patients who were referred to the consultant clinic at the department of Rheumatology, AL-Yarmouk teaching hospital, Baghdad teaching hospital from Dec. 2013 to May 2014. The diagnosis of those patients has been performed under supervision of a specialist physician in rheumatology deportment. A number of 75 Iraqi RA patients, who fulfilled the American Rheumatism association criteria for diagnosis of RA. The patients age who involved in this study ranged from 20- 67 years wasdivided into three groups 30 patients was gave Methotrexate(MTX) and 30 patients gave Etanercept (ETN), 15 patients without treatment for RA and 15 healthy persons acontrolgroup.From each participating subject, 3-5 ml of blood was obtained by venipuncture. The collected blood was transferred to a plain tube and left to clot at room temperature (20-25°C) for 15 minutes. The clotted blood was centrifuged at 2000 rpm for 15 minutes; and by then, serum was collected and distributed into aliquots of (200 µl) in Eppendorf tubes, which were frozen at

-20°C until laboratory assessments. Serum samples were collected from all study individuals to determine the seropositivity levels of certain cytokine:IL1- α and anti-CCP using enzyme linked immunosorbent assay (ELISA) and laboratory IL1-α kits used by Boster-USA Company(IL1-α) andMedipan-Germany (anti-CCP).

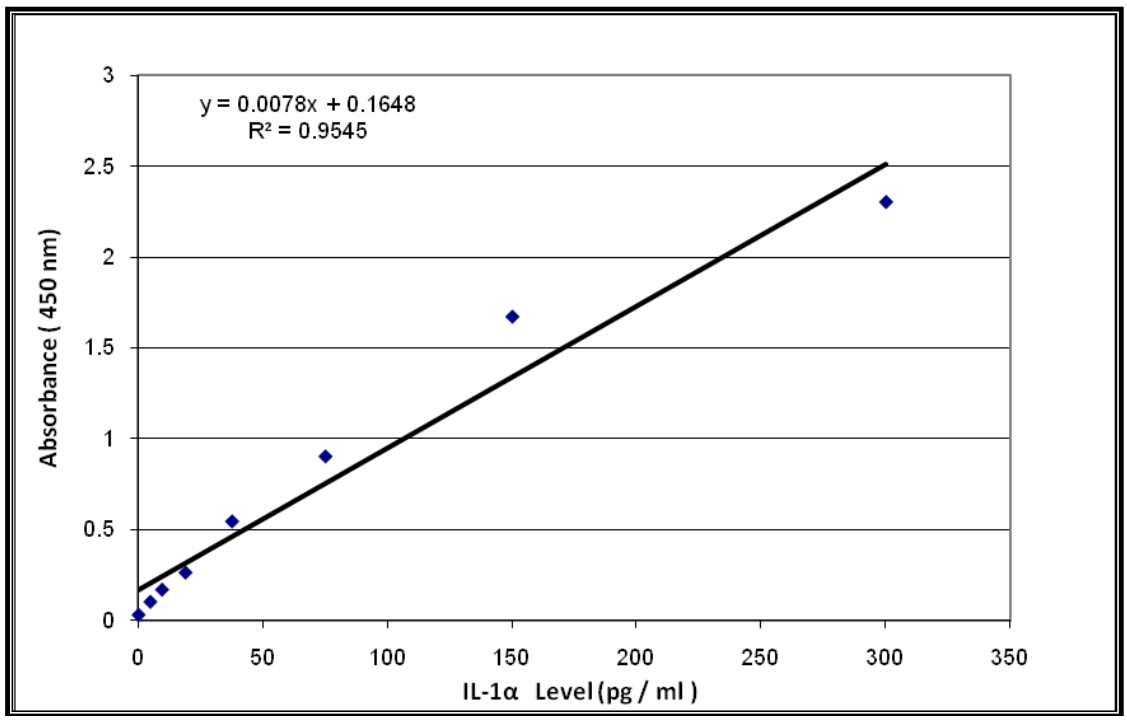

Figure 1- Standard Curve of IL- 1α Serum Level.

Statistical Analysis

The Statistical Analysis System was used to detect the effect of different factors in study parameters. Chi-square test was used to significant comparing between percentage & least significant difference –LSD test was used to significant comparing between means in this study [13].

Results and Discussion

Demographical Distribution

The demographical distributions of the studied groups according to the age were shown in table-1. The results clarified that the age was ranged between (20 – 67 years) and the mean± SE for RA was 46.16 ± 1.24.

Table 1-Mean ± SE of age in control and patients group.

Groups	Mean	SE	Range
HC	46.33	3.04	23.00-63.00
RA	46.16	1.24	20.00-67.00

RA= rheumatoid arthritis, HC= healthy control, SE= standard error.

The current study revealed that the age range for the majority of RA patients was at (41–60 years), which is confirmed by [14, 15]. The disease of RA can occur at any age, and its prevalence increases with age [16].

Anti-CCP antibodies

The results in table-2 observe a significant increase the mean of anti-ccp positivity in group III non treatment patients (86.67%) when compare with control group ($P<0.01$). The mean of anti-ccp positivity also showed a significant increase in group III non treatment patients comparing a group II

ETNwith treated patients (53.33%) and group I MTX treated patients (70%). Also the mean of anti-ccp is significant increase in group I than in group II (P<0.01). Whereas the comparison revealed between positive and negative was appeared significant variation in positive than negative in all groups of the study except group II.

Table 2-DistributionAnti-CCP level in the sera of studied group.

Groups	No. (%)	
	Negative	Positive
Control	15 (100%)	0 (0.00%)
I: MTX	9 (30.00%)	21 (70.00%)
II: ETN	14 (46.67%)	16 (53.33%)
III: Non	2(13.33%)	13 (86.67%)
Chi-square ($\chi 2$)	15.22 **	15.22 **
		** (P<0.01).

Anti-CCP: Anti-cyclic citrullinated peptide.

Anti-CCP serological marker for RA should be highly specific for the disease and be able to distinguish RA from other arthritis that mimic RA. Recently a highly specific autoantibody system described for RA, in which patients developed. Autoantibodies to citrallinated and this has resulted in the development of anti- cyclic citrallinated peptide (anti-ccp) antibody test [17].

Our results were agreement with [15] which was observed significant increase of anti-ccp in the sera of RA patients. Also the results is agreed with [14].Observe significantly Sakyi [19], increase of anti-ccp in the sera of RA patients.

A study has appeared the level of anti- ccp was significantly decreased in etanercept treated patients, also in our study the anti- ccp level was significant decrease when comparison with non-treatment patients Chen *et.al*. [18].

Interleukin- 1α: Figure-1

The results in table- 3appear that the mean of IL- 1α is significant increase in group III non treatment **(**24.57 ± 3.73 pg/ml**)** than control **(**13.69 ± 1.61 pg/ml) and group I(13.54 ± 1.16 pg/ml) and group II (13.06 ± 0.83 pg/ml). While non-significant difference between control, group I and group II.

Table 3-The level of in IL- 1α in the sera of study groups (Mean ± SE).

Groups	No.	Mean ± SE (pg/ml) 0f IL-1α
Control	15	13.69 ± 1.61 b
I: MTX	30	13.54 ± 1.16 b
II: ETN	30	13.06 ± 0.83 b
III: Non	15	24.57 ± 3.73 a
LSD value	----	4.918 **
P- value	----	0.0001

** (P<0.01).

In the present study, the level of serum IL- 1α was significantlydifferent among RA cases and control. Elevated levels of IL-1α were not commonly found in the circulation or in body fluids except during sever disease, in which case the cytokine may be released from dying cells. It is less easy to get an impression of the role of IL-1α in the pathogenesis of inflammatory disease [20, 21]. Several studies in recent years were conducted for evaluation of inflammatory cytokines such as IL-1α and

TNF-α in rheumatologic disorders including rheumatoid arthritis to find new treatment methods base to pathogenesis. These studies reveal disequilibrium between stimulatory and inhibitory mechanisms inflammatory disorder such as rheumatoid arthritis and cytokines (IL-1α and TNF-α) have a leading role in pathogenesis [6, 7]. The result isagreement with [22] who mentioned decreased the level of IL-1α in treated RA patients by MTX. These finding demonstrate that IL-1α may have a significant effect in the pathogenesis of RA and may be used as indicators of disease activity, and MTX seems to be an efficient inhibitor of cytokine production [23 - 25]. Also [26] demonstrate increased level of IL-1α in RA patients compared with control. .

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