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Evaluation of the Human Pulmonary Activation-Regulated Chemokine (CCL18/PARC) and Alkaline Phosphatase (ALP) Levels in Iraqi Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is one of the chronic inflammatory autoimmune diseases which occurs as a result of unknown reasons. This study was conducted at Baghdad Teaching Hospital/City of Medicine, where blood samples were taken from 60 Iraqi patients with RA (49 females and 11 males) and these patients were matched by age and sex with 20 healthy controls (16 females and 4 males). Patients with RA were diagnosed by a consultant rheumatologist according to ACR / EULAR criteria in 2010. In this study the patients were divided into **four** groups as follows; the first group consisted of 12 patients treated with methotrexate (MTX), the second group consisted of 10 patients treated with etanercept, the third group consisted of 18 patients treated with a combination of MTX, etanercept and prednisolone, the fourth group consisted of 20 patients treated with MTX and etanercept. Enzyme linked immunosorbent assay (ELISA) was used to detect CCL18/PARC antibodies, while a spectrophotometer (Humalyzer2000) was used for the measurement of alkaline phosphatase (ALP). Serum levels of CCL18 / PARC showed a significant increase in RA patients compared with healthy controls ($p \leq 0.001$). The levels of CCL18/PARC showed a significant correlation with disease activity (CDAI), except in RA patients treated with etanercept. There was also no significant correlation between CCL18/PARC and erythrocyte sedimentation rate (ESR). The results showed a significant increase in serum levels of alkaline phosphatase (ALP) was recorded in RA patients (with treatment) as compared to healthy controls ($p \leq 0.001$).

Keywords: Rheumatoid arthritis, Human Pulmonary Activation Regulated Chemokine, Alkaline phosphatase.

تقييم مستويات التنشيط الرئوي المنتظم للانسان(CCL18 / PARC) والانزيم المزيل للفوسفات القاعدي (ALP) في المرضى العراقيين المصابين بألتهاب المفاصل الرثوي

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الخلاصة

التهاب المفاصل الرثوي : هو أحد أمراض المناعة الذاتية الالتهابية المزمنة الذي يحدث نتيجة لأسباب غير معروفة. أجريت هذه الدراسة في مستشفى بغداد التعليمي / مدينة الطب ، حيث تم أخذ عينات دم من 60 مريض عراقي مصاب بالتهاب المفاصل الرثوي (49 إناث و 11 ذكور) وتمت مطابقة هؤلاء المرضى حسب العمر والجنس مع 20 شخص ضمن مجموعة الضوابط الصحية (16 إناث و4 ذكور). تم تشخيص المرضى الذين يعانون من التهاب المفاصل الرثوي من قبل استشاري أمراض الروماتيزم وفقا لمعايير ACR / EULAR في عام 2010. في هذه الدراسة تم تقسيم المرضى إلى اربع مجاميع على النحو التالي; المجموعة الأولى تتألف من 12 مريضاً عولجوا بـ ميثوتركسيت ، المجموعة الثانية تتألف من 10 مريضاً عولجوا بـ ايتانرسيبت المجموعة الثالثة تتكون من 18 مريضاً عولجوا مع (ميثوتركسيت ، ايتانرسيبت و بريدنيزولون) ، المجموعة الرابعة تتألف من 20 مريضاً عولجوا بـ (ميثوتركسيت و ايتانرسيبت). في هذه الدراسة، تم استخدام مقياس الانزيم المناعي المرتبط (ELISA) للكشف عن الأجسام المضادة CCL18/ PARC وايضا تم استخدام مطياف الضوء المرئي (Humalyzer2000) لقياس ALP . أظهرت النتائج وجود ارتفاع معنوي ذات دلالة احصائية في مستويات CCL18/PARC في مرضى التهاب المفاصل الرثوي (المعالجين) مقارنة مع الضوابط الصحية ($P \leq 0.001$). أظهرت النتائج وجود علاقة إيجابية بين تركيز CCL18/PARC ونشاط المرض في مرضى التهاب المفاصل الرثوي وايضا عدم وجود علاقة معنوية بين مستوى CCL18/PARC في مرضى التهاب مفاصل الرثوي الذين عولجوا بـ ايتانرسيبت و نشاط المرض وكذلك اظهرت النتائج عدم وجود فرق معنوي بين مستويات CCL18/PARCوESR. اظهرت النتائج وجود ارتفاع معنوي ذات دلالة احصائية في مستويات (ALP) في مرضى التهاب المفاصل الرثوي (المعالجين) مقارنة مع الضوابط الصحية ($P \leq 0.00\ 1$) .

Introduction

Rheumatoid arthritis (RA) is one of the types of autoimmune diseases of chronic inflammation, where the immune system attacks the endothelial cells of the joints causing chronic inflammation of the joints. Although RA affects the joints mainly and causes pain and damage in bones and cartilage, it has other effects on the lungs, eyes, heart and skin [1]. The main cause of RA is not yet known and it is also believed that environmental, genetic and aging factors have a role in etiology [2]. In a previous study, a statistical analysis was conducted by sex, age, year, cause and geography of many diseases, including rheumatoid arthritis from 2005 to 2015. It was found that RA affected up to about 24.5 million people [3]. The prevalence of RA worldwide is between (0.5 - 1%), it also affects people of age 30 – 50 years as well as children 16 years and above, and RA affects women 3 times more than men. The symptoms of RA appear in some patients in varying periods of time that may be from days to weeks or months and could even take several years. The passage of all this time without diagnosis and treatment leads to permanent deformations, including tendon rupture and erosion of cartilage and bones. The symptoms of RA are accompanied by pain and swelling of the joints, loss of weight, appearance of red circles under the skin, inability to breathe, pain in the chest and dry eye [4]. Increased RA disease activity leads to the destruction of cartilage and bones in the joints, also different types of treatments are used to reduce both disease progression and symptoms only because the effectiveness of drugs is limited and it does not eliminate RA [5-6]. Chemokine (CK) is one of the types of cytokines with a low molecular weight (6–14 k Da) and it's known by other names including; Human CCL-18 (CC-chemokine ligand-18) Pulmonary and Activation-Regulated Chemokine (CCL18/ PARC), Macrophage Inflammatory Protein-4 (MIP-4), Alternative Macrophage Activation-associated CC Chemokine 1 (AMAC-1) and Dendritic Cell Derived Chemokine 1 (DCCK1)[7]. Chemokine has important roles in determining the response of white blood cells and the assemblage of inflammatory cells in the site of inflammation. In addition, the excretion of CCL18/PARC is performed through the innate immune system. Although CCL18/PARC exerts chemical functions in the innate immune system, these functions are mainly related to the induction of the adaptive immune system [8]. The gene of CCL18/PARC is most analogous to that of CCL3. CCL18/PARC is present within several macrophages (MIPs), where it is located on the chromosome 17 [9]. Alkaline phosphatase (ALP) is one of the enzymes found in blood plasma and has a high molecular weight (140kDa). The effectiveness of ALP is increased in alkaline pH (9 - 10.5) [10]. Alkaline phosphatase

is found in some tissues of the body, mainly in the bones and liver and in a few amounts in the kidney, placenta, and bowel [11]. Besides that, ALP contains a group of enzymes that include; the isoenzyme produced by the bone (isoenzyme ALP-1) and the isoenzyme produced by the liver (isoenzyme ALP-2). The function of these enzymes is to facilitate the transport of metabolic products through membranes and lipids. ALP has a low hydrolysis group, which provides it with the ability to decompose a large group of phosphate esters by forming phosphate and alcohol ions. In addition, it is worthy to note that levels of ALP in the blood serum are high as a result of inflammation that occurs in the joint, which leads to an acceleration in the rate of bone turnover [12-13].

The aim of this study is to evaluate the levels of CCL18 / PARC and ALP in Iraqi patients with RA (with treatment), as compared with healthy controls, and to assess the role of CCL10/PARC as a possible marker of disease activity.

Materials and Methods

This study was conducted at Baghdad Teaching Hospital/City of Medicine, where blood samples were taken from 60 Iraqi patients with RA (49 females and 11 males) and these patients were matched by age and sex with 20 healthy controls (16 females and 4 males). Patients with RA were diagnosed by a consultant rheumatologist according to ACR / EULAR criteria in 2010. Blood samples were collected from September 2018 to January 2019. The medical history was collected from every patient and included the age, sex, chronic disease, duration of smoking, duration of the disease, duration of treatment, type of treatment (methotrexate, etanercept, and prednisolone), and the measurement of Clinical Disease Activity Index (CDAI). The disease activity was measured using the following equation;

CDAI= Swollen 28-Joint Count + Tender 28-Joint Count + Patient Global disease Activity + Evaluator's Global disease Activity.

After that, patients were divided into **four** groups as follows; the first group consisted of 12 patients treated with MTX, the second group consisted of 10 patients treated with etanercept, the third group consisted of 18 patients treated with a combination of MTX, Etanercept and prednisolone, the fourth group consisted of 20 patients treated with MTX and etanercept. Five milliliters of blood were withdrawn from patients with RA and healthy controls. The blood samples were placed in test tubes (Gel Tube) and centrifuged at 3000 rpm for 10 minutes. After that, the serum was placed in a small tube (Eppendorf tube) and stored at temperature of ˉ40°C. The serum was used to determine CCL18 / PARC level using the Sandwich-ELISA principle (Elabscience). The remaining serum was used to measure ALP ~~levels~~ **activity** by spectrophotometer (Humalyzer2000). In this study, patients with migraine, epilepsy, and thyroid and heart diseases were excluded.

Statistical analysis

Data analysis was performed using SPSS statistical program (Version/11.5; SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means. Data were presented as mean ± Stander Deviation. Pearson's correlation (r- coefficient) was used between CCL18/PARC, disease activity and ESR. A p-value of ≤ 0.05 was considered statistically significant.

Results and Discussion

Human Pulmonary Activation Regulated Chemokine CCL-18/ PARC levels

In this study, the results showed a significant increase in serum CCL18 / PARC levels in RA patients as compared with healthy controls (Table-1). The cause of the high level of CCL18/PARC in RA patients was due to the infiltration of inflammatory cells such as stem cells, T cells, B cells and connective cells into the joints, causing the white blood cells to move to the site of inflammation and release cytokines that attacks the cells and releases other destructive substances. Over time, inflammation increases on the synovial membrane and pain occurs in the joints, associated with cartilage damage. It's also known that the synovial fluid and synovial tissue in RA patients contain high concentrations of several chemokines [14]. The results of this study were agreed with the previous studies [15-16], where the previous study showed a significantly increased in the serum levels of CCL18/PARC in RA patients compared with osteoarthritis patients (OA), and healthy controls [15] and also the previous study found a significantly increased in the serum levels of CCL18/PARC into the synovial fluid of RA patients compared with healthy controls [16].

Table 1- Serum levels of CCL18/PARC in RA patients (with treatment) and healthy controls

Groups	CCL18/PARC Mean ± SD (pg/mL)	P. value
Healthy controls	1144.92 ± 386.16	
RA patients with MTX	8327.54 ±548.1	0.001
RA patients with Etanercept	8330.79 ± 1117.96	0.001
RA patients with MTX – Etanercept	8589.13 ± 650.57	0.001
RA patients with MTX –Etanercept – Prednisolone	8649.60 ± 784.59	0.001

MTX: Methotrexate, RA: Rheumatoid arthritis, SD: Stander deviation,

The results in Figure- 1 show that the monotherpay with MTX for RA patients caused reduction in the level of CCL18/PARC compared to those of the combination therapy, although the reduction did not reach a statistically significant level. The mean values of serum CCL18/PARC levels using monotherpay with MTX was 8327.54 ± 548.1pg/mL, monotherpay with Etanercept was 8330.79 ± 1117.96pg/mL, combination therapy with MTX and etanercept was 8589.13 ± 650.57pg/m), and combination therapy with MTX. etanercept, and prednisolone was 8649.60 ± 784.59pg/mL).

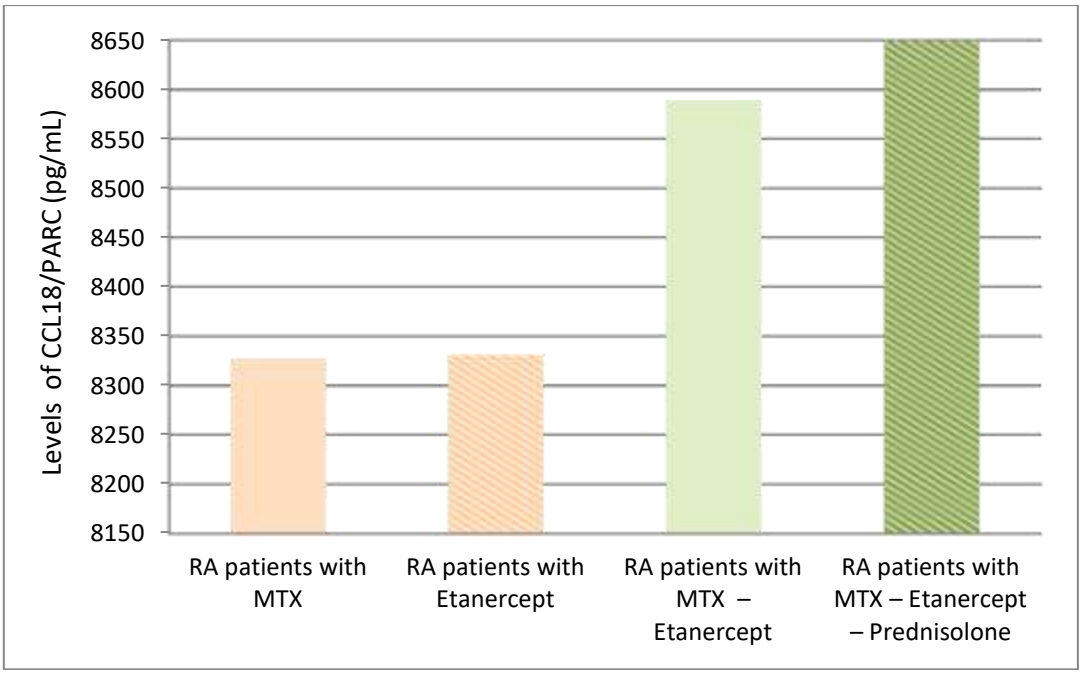

Figure 1-Serum levels of CCL18/PARC in RA patients receiving monotherapy and combination therapy

Correlation between CCL18/PARC concentrations in RA patients (with treatment) and disease activity (CDAI)

Data in Table-2 show a significant positive correlation between CCL18/PARC in RA patients (with treatment) and CDAI, while the correlation between CCL18/PARC in etanercept –treated RA patients and CDAI was non-significant. **The results of the study were agreed with the previous studies [17-18], where the previous studies showed a positive correlation between CCL18/PARC levels and disease activity.**

Table 2- Correlation between CCL18/PARC and disease activity CDAI

Groups	R	p- value
RA patients with MTX	0.72	0.005
RA patients with Etanercept	0.70	0.106 (NS)
RA patients with MTX – Etanercept	0.80	0.001
RA patients with MTX – Etanercept – Prednisolone	0.81	0.001

MTX: Methotrexate, RA: Rheumatoid arthritis, NS= Non-Significant, R=Pearson Correlation

Erythrocyte Sedimentation Rate (ESR)

In this study, the results showed a significant increase in serum levels of ESR in RA patients (with treatment) as compared with healthy controls (Table-3). Erythrocyte Sedimentation Rate is one of the methods used to measure the level of the inflammation in the patient and the extent of stability of red blood cells. This study is consistent with a previous study which showed a significant increase in ESR levels in RA patients compared to controls [19].

Table 3- Serum levels of ESR in RA patients and healthy controls.

Groups	ESR Mean ± SD (mm/hr)	P. value
Healthy Controls	8.70 ± 4.68	
RA patients with MTX	36.23 ± 19.99	0.001
RA patients with Etanercept	49.60 ± 28.47	0.001
RA patients with MTX – Etanercept	43.65 ± 31.57	0.001
RA patients with MTX – Etanercept – Prednisolone	35.17 ± 24.73	0.001

MTX: Methotrexate, RA: Rheumatoid arthritis, SD: Stander deviation, ESR: Erythrocyte Sedimentation Rate

Correlation between levels of CCL18/PARC and ESR in RA patients

Data in Tables-3 and 4 show a non-significant correlation between CCL18/PARC and ESR levels in RA patients. The results of this study agree with those of a previous study, which shows a non-significant correlation between CCL18/PARC and ESR levels [15].

Table 4- Correlation between serum levels of ESR and CCL18/PARC in RA patients

Groups	R	p- value
RA patients with MTX	0.13	0.865(NS)
RA patients with Etanercept	0.24	0.677 (NS)
RA patients with MTX – Etanercept	0.10	0.623 (NS)
RA patients with MTX – Etanercept – Prednisolone	0.20	0.523 (NS)

MTX: Methotrexate, RA: Rheumatoid arthritis, NS= Non-Significant, R=Pearson Correlation

Alkaline Phosphatase (ALP)

In this study, the results showed a significant increase in ALP levels of RA patients (with treatment) as compared with the healthy controls (Table-5). Increased serum ALP in patients with RA may be due to the change in the effectiveness of enzymes as a result of the damages in the bones and liver, which causes the release of these enzymes with more effectiveness than that observed in the normal conditions .The present study was agreed with a previous study. Previous study indicates the presence of a significant increase in serum levels of ALP in RA patients compared to healthy controls.

The authors explained their results by an ALP increase due to changes in the small joints and wrist joints. The compensatory mechanisms were reported to have an important role in restoring the deformed bones and, therefore, the activity of ALP increases [20].

Table 5- Serum levels of alkaline phosphatase in RA patients (with treatment) and healthy controls

Groups	ALP Mean ± SD (U/l)	P. value
Healthy Control	152.57 ± 43.28	
RA patients with MTX	296.95 ± 80.56	0.001
RA patients with Etanercept	305.61 ± 90.82	0.001
RA patients with MTX + Etanercept	273.99 ± 85.34	0.001
RA patients with MTX – Etanercept – Prednisolone	268.86 ± 75.64	0.001

MTX: Methotrexate, RA: Rheumatoid arthritis, SD: Stander deviation

Limitation of the study

The limitations of this study are small sample size and the total sample 80, which was divided into 60 RA patients and 20 healthy controls as well as not available time to measure other parameters signs that have a link to the inflammation of RA

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