



## Estimation of Levels of Interleukin-1 beta and Interleukin-10 in Sera of some Iraqi Patients with Chronic Rheumatoid Arthritis

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### **Abstract**

Serum samples were obtained from patients suffering from chronic Rheumatoid Arthritis (RA) disease. They were divided into three groups; 20 patients with RA taking biological treatment Etanercept (Enbrel), 20 patients with RA not taking biological treatment (disease modifying anti-rheumatic drug) and 10 people as healthy control group. The ages of these patients and control group were between 46-50 years old; one male and 19 female in each group. The samples were used for measuring the levels of Interleukin (IL)-1 Beta and Interleukin-10 using the Enzyme Linked Immunosorbent Assay technique. Both interleukins were at their highest levels in the group of RA patients treated with biological treatment followed by the RA patients group that were not treated with biological treatment and the lowest levels were in the healthy control group.

**Keywords:** Rheumatoid arthritis, Interleukin-1, Interleukin-10, Enzyme Linked Immunosorbent Assay (ELISA) technique, biological treatment.

## تقدير مستويات الانترلوكين - ١ بيتا والانترلوكين - ١٠ في مصل بعض المرضى العراقيين المصابين بالتهاب المفاصل الرثوي المزمن

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### **الخلاصة**

تم جمع عينات المصل من المرضى المصابين بالتهاب المفاصل الرثوي المزمن. وتم تقسيم هذه العينات الى ثلاثة مجتمعات: المجموعة المصابة بالتهاب المفاصل الرثوي المزمن والتي تم علاجهم بالعلاج البالبوليوجي اينترسيبيت (إينبريل) والمكونة من ٢٠ مريض، المجموعة المصابة بنفس الالتهاب لكن بدون اخذ العلاج البالبوليوجي (فقط باستخدام الأدوية المغيرة للمرض ضد الروماتويدي) والمكونة من ٢٠ مريض والاصحاء كمجموعة سيطرة فقط ١٠ اشخاص. كانت الاعمار المستهدفة في هذه الدراسة بين ٤٦-٥٠ سنة وكل مجموعة كانت مؤلفة من واحد ذكر و ١٩ انثى. استخدمت طريقة مقياس امتصاصية الانزيم المرتبط (ELISA) لقياس مستويات الانترلوكينات حيث وجد ان مستوى الانترلوكين - ١ بيتا والانترلوكين - ١٠ كان أعلى في مجموعة المرضى المصابين بالتهاب المفاصل الرثوي المزمن والذين تم علاجهم بالعلاج البالبوليوجي

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وثلثها مجموعة المرضى المصابين بنفس الالتهاب لكن بدون العلاج البالغوجي بينما كانت مستوياتهم منخفضة في مجموعة الأصحاء السيطرة.

## Introduction

A well-known autoimmune disease that is characterized by systemic complications, early mortality and progressive disability is RA. The causative factors are not known with a careful diagnosis has to be made. But, the knowledge of the disease pathogenesis has led to much recent therapeutics with low incomes. They can include series of factors such as genotyping, chance, and environment. RA is also characterized by hyperplasia, inflammation of synovia, RA and ACPA antibodies production, cartilage and bone deformity, and many systemic signs such as psychological, skeletal, pulmonary, and cardiovascular disorders [1].

RA also involves increased levels of acute phase proteins and hyper-gamma-globulinemia. The mononuclear cells are found with the synovial membrane and then the T-lymphocytes are activated to stimulate rheumatoid factor production by B cells. Essential role of synoviocytes in this process too which are present tissue damage site and can produce proteases that cleave cartilage and many other inflammatory mediators. These include interleukin (IL)-1 and Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) which can destroy the inflamed joint by activation of synoviocytes to produce prostaglandins and proteases [2]. Raised levels of IL-1 concentrations in plasma in RA patients can be associated with disease activity including pain result and morning stiffness duration while their levels in synovial fluids are high too, ten times more than those suffering of osteoarthritis (OA) or other non-inflamed joint disorders [3].

The usual cellular sources of IL-10 in RA synovia are macrophages especially T cells. Studies showed ex-vivo cultures of mixed synovial cells with lymphocytes produced IL-10 and IL-1, IL-6 and IL-8. These results point out the role of IL-10 in inflammation [4]. Its levels in serum of RA patients are high as opposed to that of healthy control. These levels are not related to disease activity but with RF titers in serum [5]. In the last 20 years, there was a great progress in the treatment of RA which involved the use of disease-modifying anti-rheumatic drugs (DMARDs) and the improvement of immune therapies specific against molecules and cells vital in the immune-pathogenesis of RA. Moreover, rheumatologists have been searching for new treatments. Specific immune response features have been targeted after a decade of laboratory work. The production of targeted biological therapies to block cytokines such as TNF has been one of the major improvements in medicine in the last 20 years [6]. Biologics, the recent generation of anti-rheumatic drugs, have new molecular mechanisms against cytokines and cells of joint eruption and inflammation. They involve agents against TNF: etanercept, infliximab and adalimumab; agents against IL-1: anakinra; rituximab, the anti-B cell antibody; and abatacept, down-regulator of T cell [7].

## Aim of this study

The aim of this study is the estimation of the levels of IL-1 $\beta$  and IL-10 in some of Iraqi patients that were treated with a biological agent and those treated with non-biological agents and comparing these levels with that of the healthy subjects.

## Materials and Methods

A total of 95 blood samples were collected from patients suffering from chronic RA attending the rheumatology clinic of Baghdad Teaching Hospital in Iraq from September 2016 to April 2017. Medical history was collected from each patient (age, gender, address, symptoms of disease, medication, other related diseases, number and types of surgeries, smoking status, and laboratory tests).

The patients were divided into three groups as follows: first group was composed of 20 patients did not receive biological treatment; second group of 20 patients treated with biological treatment Etanercept and third group of 10 healthy persons (controls) that did not suffer from chronic RA. Each group was composed of one male and 19 female. The ages were between 46-50 years old. Ten milliliters of blood samples were divided into two tubes according to the tests; 5 ml of blood samples were left to clot at room temperature for 10-15 minutes and then centrifuged at 2500 rpm for 15 minutes. The resultant serum was refrigerated by freezing at (-20 °C) until it was used to determine cytokine levels using Sandwich-Enzyme Linked Immunosorbent assay (ELISA) technique according to manufacturing company (Elabscience) and the optical density used was 450nm; while the remaining blood was used in other experiments such as flow cytometry and gene expression.

## Results and Discussion

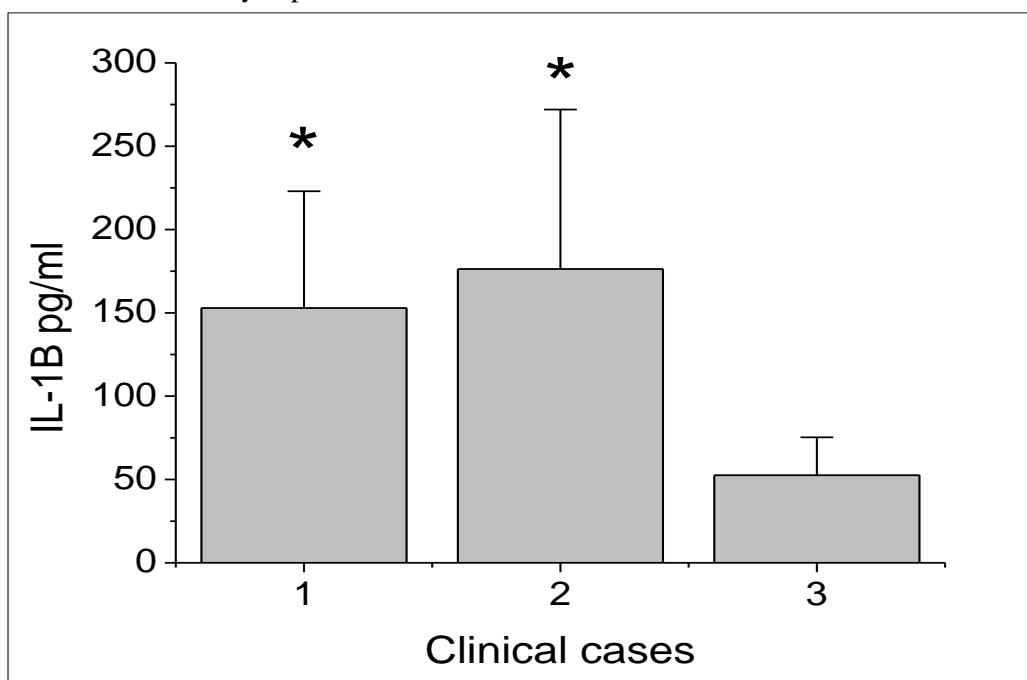
Results showed that the percentages of RA in females from 95 individuals were 85 (89%) more than in males 10 (11%). The gender difference in incidence, the increasing incidence in females in the age groups approaching menopause, as well as a shift in the incidence toward more elderly patients in females suggest that hormonal factors are involved in the pathogenesis [8] and this coincides with previous studies done in Iraq conducted by Al-Rawi *et al.*, 1978 which showed that the reason could be related to the higher number of females compared to males due to several wars, and it could be due to hormonal factors such as estrogen which affects the function of T-lymphocytes [9].

The mean age of patients was in the fifth decade; this is in agreement with other studies which showed that RA affects usually people above 40 years old. This is in fact caused by many reasons that depress immunity as stress, thymic depression, and longer duration of exposure to environmental antigens that cause stimulation of auto-reactive immune cells [10].

The medical history of this study revealed that 37% of patients have also suffered from hypertension, 22% diabetes, 17% other cases including tuberculosis, urinary tract infection, psoriasis and others. Rheumatoid arthritis associates with excessive morbidity and mortality from cardiovascular disease (CVD) which may be due to multiple causes. Several risk factors, such as hypertension, smoking, dyslipidaemia, (as defined by National Cholesterol Education Program) and insulin resistance are thought to be more prevalent in RA and may be important contributors [11].

### Levels of IL-1 $\beta$ in sera samples

In serum of each group level of IL-1 $\beta$  has been measured. Figure-1 shows the highest level of IL-1 $\beta$  was found in sera of patients with RA who treated with biological treatment ( $176.3 \pm 96$  pg/ml), followed by non-biological treated group ( $152.9 \pm 70$  pg/ml). Significant difference was found between levels of IL-1 $\beta$  in sera of patients with RA who none treated with biological treatment ( $P < 0.05$ ) and healthy control group ( $52.5 \pm 22.7$  pg/ml). Similar results was observed between level of IL-1 $\beta$  in sera of patients with RA who received biological treatment and healthy control group ( $P < 0.05$ ). It was observed that the level of IL-1 $\beta$  in patients received biological treatment bit higher than level of IL-1 $\beta$  not received biological treatment that does not mean the biological treatment elevates IL-1 $\beta$  but the patients that received biological treatments almost attributed high severity of disease and these group of patients almost specified by physician to get biological treatment because this kind of medicine is very expensive.

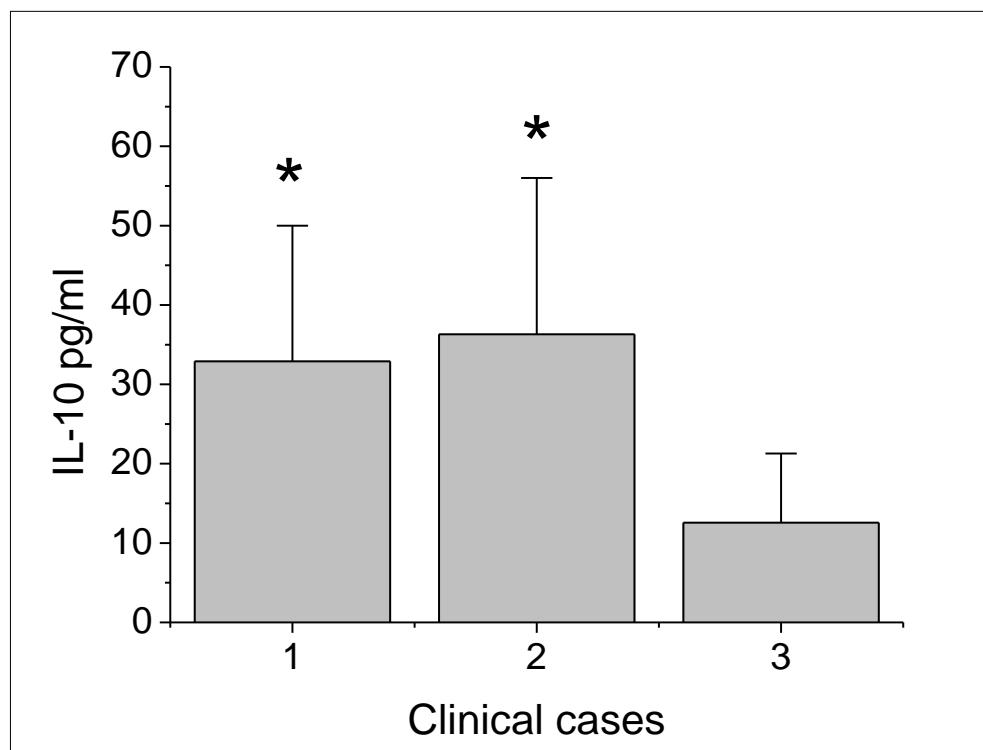


**Figure 1**-Level of IL-1 $\beta$  in sera of different groups of patients with RA and healthy control. 1, Non biological treated group; 2, Biological treated group; 3, Healthy control group. Asterisks indicate a significant difference from healthy control group.  $P < 0.05$  considered to be significant difference.

Activated macrophages are the main cellular sources of cytokines in serum. Significant levels of IL-6 are produced by endothelial cells but low amounts of IL-1 and TNF  $\alpha$  are produced [12]. Although many researches have been conducted, the RA pathogenesis remains speculative. Many pro-inflammatory cytokines are stimulated in the RA inflamed joints. The master cytokines are IL-1 $\beta$  and TNF- $\alpha$  in inflammation and in structural disruption. In one study, raised levels of IL-1 $\beta$  have been implicated in RA patients [13]. While in a different study before taking anti-rheumatic therapy, significant levels of IL-1 $\beta$  found in serum of 70 from 32 patients ( $> 10$  pg/ml) while it was (0.35pg/ml) in serum of untreated RA patients . In the same study, a significant correlation was observed between the serum IL-1 $\beta$  levels at 6 months showing high levels of IL-1 $\beta$  before and even after 6 months of therapy [14].

#### Levels of IL-10 in sera samples

Serum level of IL-10 has been measured in sera of different groups of patients with RA and healthy control group as in Figure-2.



**Figure 2**-Level of IL-10 in sera of different group of patients with rheumatoid arthritis and healthy control. 1, Non biological treated group; 2, Biological treated group; 3, Healthy control group. Asterisks indicate a significant difference from healthy control group.  $P < 0.05$  considered to be significant difference.

It shows the highest level of IL-10 was found in sera of patients with RA treated with biological treatment ( $36.3 \pm 19$  pg/ml), followed by non-biological treated group ( $32.9 \pm 17$  pg/ml). Significant difference was found between levels of IL-10 in sera of patients with RA who none treated with biological treatment ( $P < 0.05$ ) and healthy control group ( $12.5 \pm 8.72$  pg/ml). Similar results was observed between level of IL-10 in sera of patients with RA who received biological treatment and healthy control group ( $P < 0.01$ ).

The sources of IL-10 are human B cells, monocytes, and T cells. Its function in humans in a cytokine synthesis inhibitor (CSIF) which inhibit IL-4, IL-5, and interferon (IFN)- $\gamma$ . IL-10 also has potent anti-inflammatory properties [15]. It inhibits the synthesis of pro-inflammatory cytokines such as TNF- $\alpha$  and IL-1 [16].

In another study, serum IL-10 level was ( $4.32 \pm 1.87$  pg/ml) in RA patients as opposed to ( $5.87 \pm 3.85$  pg/ml) in osteoarthritis patients and normal was ( $3.48 \pm 2.34$  pg/ml).after three months of therapy, serum IL-10 decreased from  $4.32 \pm 1.87$  pg/ml to  $3.46 \pm 2.08$  pg/ml. Levels of IL-10 were elevated in

synovial fluid of patients of early RA but not in serum in comparison to controls in the same study. After 3 months of methotrexate and chloroquine combination therapy, serum IL-10 declined in almost all the responders [17]. As a response to inflammation, IL-10 increases to keep it steady but it falls short of this. IL-10 also has been shown to be produced late after pro-inflammatory cytokine release which proves it to be a suppresser of the immune process. IL-10 has been proven to down regulate and up-regulate many immune functions [18].

### Conclusions

From the current study, it has been shown that levels of IL-1 $\beta$  and IL-10 were highest in patients suffering of RA disease treated with biological treatment and they were also high in the non-biologically treated patients as opposed to healthy control group and this correlates with many other studies as these cytokines play an important role as immune mediators in RA disease and treatment of this disease depends on the imbalance between these mediators. This difference among cytokine concentrations shows the complicated integration between these cytokines and their regulatory functions. Balancing the anti- and pro-inflammatory cytokines clarify the degree and duration of inflammation. Recently, the use of such biological drugs to neutralize these cytokines or block their receptors is increasing and this study can reflect the effects of the biologics which can be used as future therapy.

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