



## Evaluation of some Interleukins in Sample of Iraqi Female Patients with Rheumatoid Arthritis

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**Abstract:** Rheumatoid arthritis, commonly abbreviated as RA, an autoimmune systemic disease, is characterised by symmetric inflammatory polyarthritis. Rheumatoid arthritis mostly affects joints that are smaller, such as those in the feet and hands but it can also impact larger joints. Cytokines play an important role in the etiopathogenesis of rheumatoid arthritis during the course of the disease; inflammatory responses and joint degradation are probably caused by cytokines. The following research was carried out on 100 female RA patients and 100 female healthy control with mean ages of patients and controls (37.44±23.10 and 49.74±9.775) respectively. Erythrocytes Sedimentation Rate (ESR), Rheumatoid Factor (RF), Serum C-reactive Protein (CRP), and Anticyclic Cetrolinated Peptide (Anti-CCP) were investigated in this study with concentrations (37.44±23.10 mm/h , 22.72±1.44 IU/ml, 16.56±0.842 IU/ml and 28.09±6.68 EU/ml), respectively. This study was examined the interleukins levels (IL-34, IL-36 and IL-37) during the development of Rheumatoid Arthritis (RA) in Iraqi female. The differences in the levels of interleukins in the serum of patients as compared to healthy controls have been included in the study. The study concluded that the serum level of (IL-36 and IL-37) appeared a higher significant differences ( $p < 0.0001$ ) in patients compared with the healthy control, (11.45±3.3ng/mL vs 7.9±2.1 ng/mL) and (111.6±21.9 ng/mL vs 78.7±19 ng/mL) respectively. It was concluded that the rheumatoid arthritis patients' serum levels of (IL-34) were markedly lower than those of the controls (130±60.7 ng/mL vs 185.9±31.6 ng/mL) ( $P < 0.001$ ).

**Keywords:** autoimmune disease, interleukins, rheumatoid arthritis, il-34, il-36, il-37.

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### Introduction

Rheumatoid arthritis (RA) is a widespread autoimmune disease. Progressively inflames the joints and surrounding tissues, eventually resulting in irreparable joint damage (1). It is estimated that women are affected up to three times more frequently than men, with an estimated frequency of less than 1%. In order to alleviate symptoms and reduce joint swelling for newly diagnosed RA patients non-steroidal anti-inflammatory drugs (NSAIDs) used to be the suggested therapy for reducing symptoms and joint swelling (2). Course of treatment to reduce

symptoms and joint swelling (2). The synovial joint is where the pathogenic process of RA is most concentrated, where immune cells have invaded and released inflammatory mediators and matrix-degrading enzymes together with synovial fibroblasts, which in turn contributed to bone erosion and cartilage damage (3). As a result of the release of pro-inflammatory cytokines and other pro-inflammatory elements, joints are damaged and rendered disabled (4). But even though the actual etiology and pathogenesis of RA are still unknown, it is well established that inflammatory mediators are crucial in

promoting synovial cell activation which leads to arthritis's inflammation and joint destruction. Numerous studies has demonstrated elevated levels of a number of pro-inflammatory mediators, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and IL-1 $\beta$ , in the serum and synovial fluid of RA patients, which induce the migration of inflammatory cells into damaged tissues and increase joint damage during the disease process (5).

Cytokines have a crucial role in the etiopathogenesis of RA. During the course of the disease, inflammatory responses and joint degradation are probably caused by cytokines. The imbalanced regulation of cytokines results in an increase in the production of proinflammatory cytokines and a reduction in the production of inhibitory cytokines, both of which contribute to the chronic inflammatory condition when combined (6). In autoimmune diseases, the interleukin (IL)-1 cytokine family significantly regulates the expression of genes associated to inflammation (7). This study focuses on three cytokines from the interleukin (IL)-1 family IL36, IL37 and IL38. They have immunomodulatory actions and they are a novel anti-inflammatory cytokine (8). A recent study indicated they markedly increased in serum of RA patients as well as in the synovial fluid. (9) . Furthermore, it is important to observe that IL-34 is assumed to play a role in the pathogenesis of RA through regulating molecules related to autoimmunity, including chemokines and pro-inflammatory cytokines, Recent research have demonstrated that IL-34 is also found to be expressed in synovial fibroblasts, the lining layer of synovial joints in RA patients (2).IL-36 cytokines have an important function in tissue homeostasis and inflammation (10). Normal IL-36 signaling improves

tissue homeostasis by facilitating the healing of wounds and repairs tissue, whereas a number of inflammatory diseases have been linked to abnormally high levels of IL-36 signaling. Additionally, it may link the innate immune system and the adaptive immune system (11). IL-36 is elevated in synovium-infiltrated plasma cells of individuals with rheumatoid arthritis, promoting the production of IL-6 and IL-8 by synovial fibroblasts. The connection between IL-36 and synovitis induction could be explained by this pathway (12). IL-37 It is a novel cytokine having anti-inflammatory and immunomodulatory characteristics. It specifically reduces inflammatory and immunological responses by reducing anti-inflammatory cytokine production; accumulating evidence indicates that IL-37 expression is associated with a number of autoimmune diseases, one of which is rheumatoid arthritis (RA) (9). The concentration of the cytokine IL-37 is extremely low in normal human plasma and other body fluids, but it is much higher in RA patients' synovial fluid, serum and peripheral blood mononuclear cells (PBMCs) (13). Increased serum IL-37 levels are related with inflammatory markers such the erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), C-reactive protein (CRP) and anti-cyclic citrullinated peptide antibody (anti-CCP) (9). The current study aimed to measure the concentration of ESR, RF, CRP, anti-ccp, as well as study interleukins for patients with RA and compare them with control models.

## **Material and method**

### **Patients and controls**

One hundred of female patients with RA between the ages of 20 and 45 were included in this study, were obtained from Baghdad city (medical city, Baghdad Hospital)/ Iraq and

diagnosed RA patients on the basis of Erythrocytes Sedimentation Rate (ESR), Rheumatoid Factor (RF), Serum C-reactive Protein (CRP), and Anticyclic Citrullinated Peptide (Anti-CCP) tests. All the study participants' patients had written informed consent and the approval of the local ethics committee (CSEC/0122/0001). In this study 100 Female healthy controls were obtained from National Blood Transfusion Center with ages ranging from 20 to 40 years old.

#### Blood collection

Five millilitres of blood had been taken from every patient and control subject. The blood was placed into EDTA-free gel tubes to get serum. The serum was then clotted at 4°C for an hour and centrifuged at 2000 g for 10 minutes to determine the level of interleukins.

#### Measurement of interleukins levels

The obtained serum was stored at -20°C until analysis. Measurements of interleukins (IL-34 IL-36 and IL37) in the serum samples were performed using the enzyme-linked immunosorbent

assay (ELISA) sandwich kits (BT-Lab, China) in accordance with the manufacturer's protocols. (IL-34 Cat.No E0043Hu, IL-36 Cat.No: E7518Hu and IL-37 Cat.No:E1947Hu).

#### Statistical analysis

Version 20.0 of the Statistical Package for the Social Sciences (SPSS) was used to analyse the data (IBM, Chicago, IL). As applicable, data are given as mean  $\pm$  standard deviation or as numbers. Utilizing the student's t-test, parametric values were evaluated. Nonparametric values were examined using the Chi-square test or Fisher's exact test.

#### Results and discussion

Totally 100 RA female patients with the mean age of 49.74 $\pm$ 9.775 years and 100 female healthy control with mean age of 37.65  $\pm$  8.09 were recruited in the study. The levels of (ESR), (RF), (CRP) and (anti-CCP) were 37.44 $\pm$ 23.10 mm/h, 22.72 $\pm$ 1.44 IU/ml, 16.56 $\pm$ 0.842 IU/ml and 28.09 $\pm$ 6.68 EU/ml, respectively. Demographic and clinical features of patients are summarised in Table (1).

**Table (1): Characteristics at baseline of rheumatoid arthritis patients and healthy controls.**

Parameters	(mean $\pm$ SD)		p- value	Normal value
	Control No.=100	Patients No.=100		
Ages(years)	37.65 $\pm$ 8.09	49.74 $\pm$ 9.775	p $\leq$ 0.0001**	-
ESR mm/h	14.94 $\pm$ 4.175	37.44 $\pm$ 23.10	p $\leq$ 0.0001**	0-20
RF IU/ml	6.23 $\pm$ 5.76	22.72 $\pm$ 1.44	p $\leq$ 0.0001**	<15
CRP IU/ml	7.88 $\pm$ 4.04	16.56 $\pm$ 0.842	p $\leq$ 0.0001**	<15
Anti-CCP EU/ml	9.78 $\pm$ 4.47	28.09 $\pm$ 6.68	p $\leq$ 0.0001**	<20

Erythrocyte sedimentation rate (ESR), Rheumatoid factor, (RF), C-reactive protein, (CRP), (anti-CCP) anti-cyclic citrullinated peptide antibodies(anti-CCP).

Chronic active rheumatoid joint inflammation frequently results in

irreparable damage of and subchondral bone articular cartilage. In rheumatoid arthritis (RA), acute-phase reactants as ESR and CRP are thought to be efficient biochemical indicators for monitoring disease activity over the long term (14).

The measurement of rheumatoid factor (RF) is not diagnostically specific for rheumatoid arthritis (RA); it can be detected in various autoimmune diseases as well as non-autoimmune disorders, and even in 3-5percentage points of the healthy population (15). In our study, RA patients had elevated levels of ESR, CRP, RF, and Anti-CCP biomarkers ,While the control was within the normal range as shown in the table (1). Studies by Serdarolu et al. (16) and Vanichapuntu *et al.* (17) supported these results. They concluded that none of these biomarkers had definitely enabled them for accurately monitor the activity of rheumatoid arthritis (RA). Anti-CCP Antibody test, which was developed as a novel serological marker for Rheumatoid Arthritis (RA), was able to help in the earlier diagnosis of RA patients and changed the decisions regarding their therapy. (18). When combined with the RF test, the anti-CCP test provides a more accurate early

diagnosis of RA and has been discovered to have a prognostic value. In addition to this, it has been found that this biomarker is related with radiographic damage in RA (19).

**Serum levels of Interleukins in patients with rheumatoid arthritis**

Table (2) showed the serum levels of IL-34, IL-36 and IL-37 in both RA patients and healthy controls

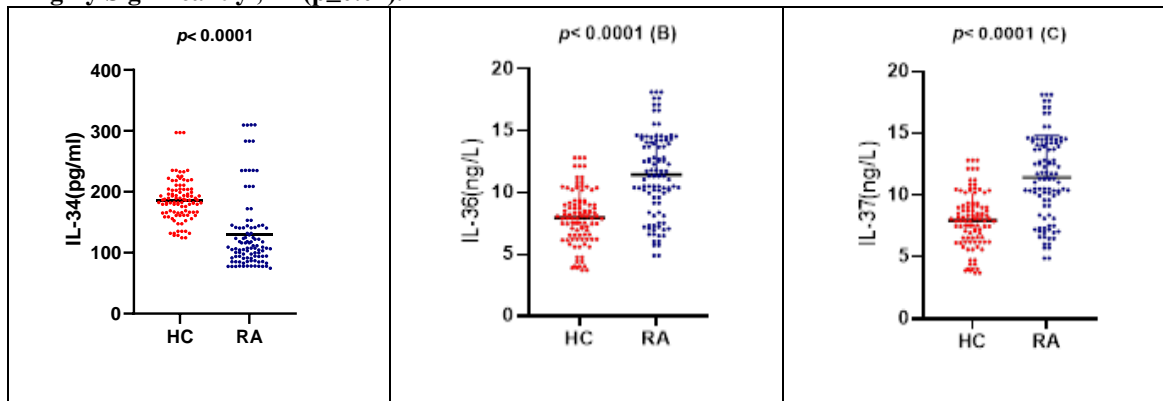
The levels of IL-34, IL-36 and IL-37, in both RA patients and healthy controls were estimated by using ELISA technique, and the corresponding levels were (130±60.7 ng/mL vs 185.9±31.6 ng/mL), (11.45±3.3 pg/mL vs 7.9±2.1 pg/mL) and (111.6±21.9 ng/mL vs 78.7±19 ng/mL), respectively. at the start, the RA group had significantly greater levels of IL-36 and IL-37 than healthy controls ( $P<0.001$ ). While RA patients had significantly lower serum levels of IL-34 than the controls, ( $P<0.001$ ) (Figure 1).

**Table (2): Serum levels interleukins in control and patients with Rheumatoid arthritis**

Interleukins	( mean± SD)		Significant	p- value
	Control No.=100	Patients No.=100		
IL-34 (ng/ml)	185.9±31.6	130±60.7	**	p≤0.0001
IL-36 (pg/ml)	7.9±2.1	11.45±3.3	**	p≤0.0001
IL-37 (ng/ml)	78.7±19	111.6±21.9	**	p≤0.0001

\*\* (p≤0.01).

Highly Significantly, \*\* (p≤0.01).



**Figure (1): serum levels of A) IL-34, B) IL-36 and C) IL-37 of rheumatoid arthritis patients and healthy controls (HC).**

The results agreed with (20, 21) which found that IL-36 and IL-37(14) were significantly greater in the RA group at the beginning compared to healthy controls.

In contrast to IL-36 cytokines, which induce inflammation, IL-37 has anti-inflammatory effects (22). This cytokine, which bind to one of the ten IL-1R family receptors and co-receptors, play a vital role in both the innate immune system and the adaptive immune system by enhancing inflammation or infection resolution. Under homeostatic situations, the production and activation of these cytokines and receptors are tightly regulated. In contrast, unregulated activation or unrestricted expression can generate or increase a pathogenic inflammatory response. The most recent IL-1 family members to be identified are IL-36 and IL-37. Their encoding genes are located on chromosome 2 and were originally cloned in 2001 (23). Even though their molecular mechanics are still not fully understood, Therapeutic benefits of targeting the IL-36 axis through skin and joint inflammation have been suggested in various research.

In this study interleukin (IL)-34 levels were observed to be reduced, and this results were agreed with previous research (24) how found that low levels IL-34 in both serum and synovial fluid, and IL-34 levels also decreased after receiving therapy.

The receptors for the cytokines interleukin (IL)-34 and macrophage colony-stimulating factor (M-CSF) are extremely similar, TNF-stimulated synovial fibroblasts elevated IL-34 levels in RA patients' synovial fluid, and they may be associated to the severity of synovitis, according to

several studies in RA patients. IL-34 could also have a role in the pathogenesis of RA. Also IL-34 may contribute in bone damage in RA, according to various studies (24, 25).

### Conclusion

Serum levels of IL-36 and IL-37 were shown to be increased in the serum of female RA patients. While serum level of IL-34 was decreased in the female RA patients compared with healthy controls. These results might consider as biomarkers in RA development.

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