

## RESEARCH ARTICLE

## The role of IL-24 as a pro-inflammatory cytokine in some Iraqi rheumatoid arthritis patients

Sattar Brissm Hassan<sup>1</sup>, Hanaa Naji Abdullah<sup>2</sup>, Khaled Yassin Zakair<sup>3</sup>

### Abstract

**Objective:** To determine the role of interleukin-24 as a pro-inflammatory cytokine among female rheumatoid arthritis patients, and its correlation with clinical parameters.

**Method:** The case-control study was conducted from April to July 2021 at the Baghdad Teaching Hospital and Yarmouk Teaching Hospital, Iraq, and comprised women aged 26-60 years in the Al-Karkh district having rheumatoid arthritis diagnosed by a rheumatologist, and healthy controls matched for age and gender. Data was analysed using SPSS 28.

**Results:** Of the 118 subjects, 76(64.4%) were patients with mean age  $45.38 \pm 1.23$  years, and 42(35.6%) were controls with mean age  $46.48 \pm 2.02$  years. Rheumatoid factor level was highly significant among the cases  $37.30 \pm 2.50$  mg/L compared to the controls  $4.91 \pm 0.53$  mg/L ( $p \leq 0.05$ ), and similar was the case with anti-cyclic citrullinated peptides  $63.04 \pm 2.82$  U/mL versus  $14.02 \pm 0.30$  U/mL ( $p \leq 0.05$ ). Serum interleukin-24 was considerably elevated in patients  $157.52 \pm 9.40$  compared to controls  $34.75 \pm 3.06$  ( $p \leq 0.05$ ). Interleukin-24 had a significant positive correlation with rheumatoid factor, and a significant negative correlation with C-reactive protein and erythrocyte sedimentation rate ( $p < 0.05$ ).

**Conclusion:** Patients with rheumatoid arthritis had higher interleukin-24 serum levels than healthy controls, indicating that interleukin-24 may be a significant marker in rheumatoid arthritis diagnosis.

**Keywords:** Protein, Antibodies, Cytokines, Arthritis, Rheumatoid. DOI: <https://doi.org/10.47391/JPMA.IQ-21>

### Introduction

Rheumatoid arthritis (RA) is an inflammatory condition that results in gradual joint deterioration and impairment. It is caused by an immunological reaction directed at the synovial joints. Chronic inflammation, immune cell infiltration, and synovial cell activation are its defining features. Even though the cause of RA is still unknown, the identification of autoantibodies and pro-inflammatory cytokines revealed that autoreactive B and T cells get activated. Interleukin-24 (IL-24) belongs to the IL-10 family and for signals it needs two receptor complexes; IL-20RA/IL-20RB and IL-20RB/IL22RA.<sup>1</sup> It has many functions ranging from controlling immune response, homeostasis of tissues, and defending the host and control of IL-24 oncogenesis. Increased levels of IL-24 and IL-20 were founded together in the plasma of RA and spondyloarthropathy patients.<sup>2,3</sup> Additionally, chronic inflammatory and autoimmune illnesses, like psoriasis, RA and inflammatory bowel disease, are observed with elevated levels of IL-24. The pathogenicity of RA has been demonstrated to induce inflammations and infiltration of immune cells. However,

<sup>1</sup>Ministry of Health, Medical City, Iraq; <sup>2</sup>Medical Laboratory Techniques, College of Health and Medical Technology, Middle Technical University, Baghdad, Iraq;

<sup>3</sup>Department of Medical Laboratory Techniques, Kut Technical Institute, Middle Technical University, Baghdad, Iraq.

**Correspondence:** Sattar Brissm Hassan

email: [sattarbrissm970@gmia1.com](mailto:sattarbrissm970@gmia1.com)

recent studies found that IL-24 has suppressive effects on immune cells like T cells, B cells, natural killer (NK) cells, and macrophages.<sup>1</sup> IL-24 has tolerogenic properties that suppress the effector actions of immune cells. IL-24 is a multifunctional cytokine, and, as a result, it has both tolerogenic and pathogenic properties. According to a recent study,<sup>4</sup> IL-24 has a suppressive role, and in some inflammatory illnesses it may have anti-inflammatory characteristics.

The current study was planned to assess how IL-24 functions as a pro-inflammatory cytokine in RA patients, and its correlation with clinical parameters.

### Patients and Methods

The case-control study was conducted from April to July 2021 at Baghdad Teaching Hospital and Al-Yarmouk Teaching Hospital, Iraq, and comprised women aged 26-60 years in the Al-Karkh district having RA diagnosed by a rheumatologist in line with the RA diagnosis criterion of the American College of Rheumatology (ACR).<sup>5</sup> The sample was raised using simple random sampling technique. From among the patients registered with the rheumatology consultation clinics. Those suffering from kidney disease, hypogonadism, metabolic bone disease, primary bone tumour or bone metastasis, osteomyelitis, and patients receiving anti-tumour necrosis factor (TNF) therapy were excluded. Healthy controls matched for age and gender

were enrolled from among non-relatives.

The study was approved by the ethics review committee of the Iraqi Ministry of Health, and the sample size was calculated using the formula:<sup>6</sup>

$$n = \frac{2(Z_{\alpha} + Z_{1-\beta})^2 \sigma^2}{\Delta^2}$$

From all the

subjects, 3ml blood samples were drawn after taking verbal consent from each of them. The samples were centrifuged at 5000rpm, and the serum was stored at -80°C until the detection of rheumatoid factor (RF) and anti-cyclic citrullinated peptides (anti-CCP) (Elabscience Biotech, China).

Erythrocyte sedimentation rate (ESR) was assessed using an automated ESR analyser, while C-reactive protein (CRP) was measured as a response to inflammation (Atlas Medical, Germany).

To measure IL-24, a quantitative sandwich enzyme-linked immune sorbent assay (ELISA) kit was used as per the instructions provided by the manufacturer (Invitrogen, China). The absorbance was read by an ELISA reader, and the results were interpreted using the standard curve (Figure).

Data was analysed using SPSS 28. Chi-square test was used to investigate the level of statistical significance associated with various correlations for non-parametric data. A t-test was used to analyse the significance of the difference in the mean values between two continuous numeric variables.  $P < 0.05$  was considered significant.

## Results

Of the 118 subjects, 76(64.4%) were patients with mean age  $45.38 \pm 1.23$  years, and 42(35.6%) were controls with mean age  $46.48 \pm 2.02$  years. Among the patients, 39(51.3%) had a family history of RF ( $p < 0.05$ ), 16(21.1%) hypertension (HTN) ( $p < 0.05$ ) and 9(11.9%) diabetes ( $p > 0.05$ ) (Table 1).

RF level was highly significant among the cases  $37.30 \pm 2.50$ mg/L compared to the controls  $4.91 \pm 0.53$ mg/L ( $p \leq 0.05$ ), and similar was the case with anti-CCP  $63.04 \pm 2.82$ U/mL versus  $14.02 \pm 0.30$ U/mL ( $p \leq 0.05$ ) (Table 2).

CRP level was significantly elevated in the patients  $29.37 \pm 2.0$  compared to the controls  $3.12 \pm 0.19$  ( $p \leq 0.01$ ). The mean ESR level among the patients was  $42.50 \pm 1.46$  compared to  $7.48 \pm 0.82$  among the controls ( $p \leq 0.01$ ) (Table 3).

Serum IL-24 was considerably elevated in patients  $157.52 \pm 9.40$  compared to controls  $34.75 \pm 3.06$  ( $p \leq 0.05$ ) (Table 4).

IL-24 had a significant positive correlation with RF ( $r = 0.282$ ,

$p < 0.05$ ), and a significant negative correlation with CRP ( $r = -0.294$ ,  $p < 0.05$ ) and ESR ( $r = -0.256$ ,  $p < 0.05$ ) (Table 5). The association of IL-24 with age and duration of disease among the patients was not significant ( $p > 0.05$ ).

**Table-1:** Characteristics of rheumatoid arthritis (RA) patients and healthy controls.

Studied parameters	RA Patients	Control	p-value
Age years	$45.38 \pm 1.23$	$46.48 \pm 2.02$	0.785
Duration of Disease	$6.31 \pm 0.48$	$0.0 \pm 0.0$	$8.22 \times 10^{-10}$
Family history			
Yes	39 (51.3)	0 (0.0)	0.000022**
No	37 (48.7)	42 (100.0)	
Morning stiffness			
Yes	76 (100.0)	0 (0.0)	$6.93 \times 10^{-23}$ **
No	0 (0.0)	42 (100.0)	
DM type II			
Yes	9 (11.9)	0 (0.0)	0.098 NS
No	67 (88.2)	42 (100.0)	
Hypertension			
Yes	16 (21.1)	0 (0.0)	0.021 *
No	60 (78.9)	42 (100.0)	
Smoking			
Yes	4 (5.3)	0 (0.0)	0.283 NS
No	72 (94.7)	42 (100.0)	

DM: Diabetes mellitus.

**Table-2:** Serum level of RF and Anti-CCP in RA patients and controls.

Autoantibodies	Mean $\pm$ SE		p-value
	Patients	Controls	
RF	$37.30 \pm 2.50$	$4.91 \pm 0.53$	$9.86 \times 10^{-10}$
Anti-CCP	$63.04 \pm 2.82$	$14.02 \pm 0.30$	$1.50 \times 10^{-14}$

RA: Rheumatoid arthritis, RF: Rheumatoid factor, CCP: Cyclic citrullinated peptides, SE: Standard error.

**Table-3:** Estimation of CRP and ESR in RA patients and controls.

Parameter	Mean $\pm$ SE		p-value
	Patients	Controls	
CRP (mg/dl)	$29.37 \pm 2.0$	$3.12 \pm 0.19$	$7.0 \times 10^{-10}$
ESR (mm/hr)	$42.50 \pm 1.46$	$7.48 \pm 0.82$	$1021 \times 10^{-21}$

RA: Rheumatoid arthritis, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, SE: Standard error.

**Table-4:** Mean of serum IL-24 in studied groups.

Cytokines	Mean $\pm$ SE		p-value
	Patients	Controls	
IL-24(pg/ml)	$157.52 \pm 9.40$	$34.75 \pm 3.06$	0.000004

IL-24: Interleukin-24, SE: Standard error.

**Table-5:** Correlation of IL-24 with clinical parameters.

Parameters	Pearson Correlation	Age	Duration	RF	CRP	ESR
IL-24	R	-0.010	0.178	-0.089	0.282*	-0.256*

IL-24: Interleukin-24 RF: Rheumatoid factor, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate.

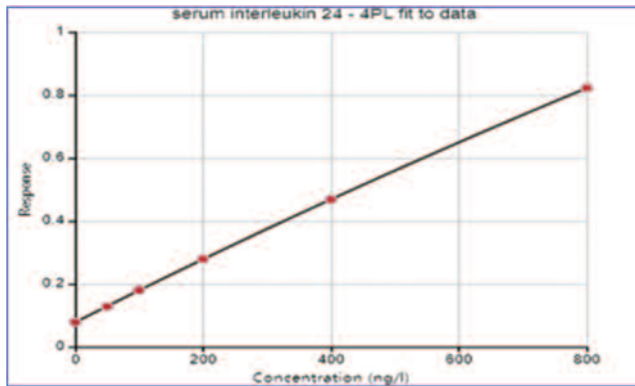


Figure: Standard curve of interleukin-24 (IL-24).

## Discussion

To the best of our knowledge, the current study is the first to look at the expression of IL-24 in sera of RA patients and to explore the association among IL-24, RF, anti-CCP and inflammatory markers. ESR and CRP have been shown to have diagnostic importance for RA.<sup>7</sup> The results of the study were in agreement with an earlier study.<sup>8</sup> The correlation between RA and anti-CCP revealed that RF was strongly related to the antibody.<sup>9</sup> These tests are helpful in the identification of the disease, including biomarkers linked to RF and positive anti-citrullinated protein autoantibodies (ACPAs) in people with chronic RA. An anti-CCP antibody is a primary indicator for the serological diagnosis of RA.<sup>10</sup>

The levels of IL-24 level in the current study were different from the levels of IL-24 documented in a previous study.<sup>10</sup> The smaller sample size for RA patients in the current study and the fact that half of the RA patients selected were active were among the reasons for the inconsistent finding. Also, different kinds and dosages of biological treatments may lead to differences in the levels of IL-24. The IL-24 found in the synovial joint of RA patients indicates that these cytokines have targeted cells at this location, but the specific cellular target subpopulation of IL-24 may be very difficult to clarify.<sup>6,11</sup> According to a study, in a psoriasis model, epidermal changes in chemokine and cytokine expression trigger not just skin lesions but also arthritis.<sup>6</sup>

This provides more evidence for the hypothesis that changes in IL-24 production outside of the synovial joint cause rheumatic disease.

In further support of this theory, IL-24 proteins were found in mononuclear cells in the current study, while a previous study observed IL-20 and IL-24 production by monocytes.<sup>8</sup>

However, no significant correlation was found in this study between anti-CCP antibody concentration and markers of disease activity ESR and CRP. These results are comparable to those stated by other reports.<sup>12</sup>

The positive relationship of IL-24 may be due to the response to RF antigens and CCPs.<sup>10</sup> The anti-CCP, CRP and ESR levels of RA patients never correlated to IL-24 levels in the current study, which used these clinical indicators to investigate the association between IL-24 levels and RA disease activity. This may be due to the paucity of relevant research, and, therefore, more studies are needed to explain the absence of this association in RA. IL-24 levels in serum and synovial fluid have been reported to be significantly elevated.<sup>10,14</sup> Because IL-24's function can change depending on the cellular source, target and stage of the immune response, the effects of IL-24 appear to be very complex.<sup>14,15</sup>

The current study has limitations. During the study period, Iraq was in the grip of the coronavirus disease-2019 (COVID-19) pandemic, and, as such, difficulties were faced in getting enough volunteers for the study. As the number of female patients visiting the hospitals was larger compared to the males, and because the latter were not very keen about participating in the study, only those who volunteered readily were selected. This could be a cause for gender bias.

## Conclusion

RA patients had higher IL-24 serum levels than healthy controls. IL-24 may be significant in RA diagnosis and in the development of the disease.

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**Conflict of Interest:** None.

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