

## Evaluation of the Changes in Some Hematological and Immunological Parameters in Patients with Rheumatoid Arthritis

Shahrazad Ahmed Khalaf, Department of Forensic Science, College of Science, Diyala University, Diyala, 32001, Iraq.

Eman A. Muhsin, Research and Technology Center of Environment, Water and Renewable Energy, Scientific Research Commission, Ministry of Higher Education and Scientific Research, 11001, Iraq.

Hamid Khalil Ali, University of Bilad Alrafidain, Diyala, 32001, Iraq.

Wedyan Yaseen Hasan, University of Bilad Alrafidain, Diyala, 32001, Iraq.

Corresponding Author email: shahrazadah.kh@gmail.com

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### Abstract

Rheumatoid arthritis [RA] is a persistent inflammatory polyarthritis condition characterized by distinct immunological, clinical, genetic, and pathological characteristics, as well as remissions and exacerbations. This study was designed to estimate some Haematological and immunological parameters of Iraqi patients with RA. The data were collected between August 2023 and May 2024 from a total of 60 patients and 30 healthy controls. A serological test was assessed, including erythrocyte precipitation rate, and CRP. Complete blood count was measured using a fully automated quantitative Samsung device. The levels of IL-1 $\beta$ , IL-2, IL-4, IL-10 cytokines were assessed by sandwich ELISA. The result of this study showed that most RA patients were female compared to males. The levels of RF, CRP, and ESR test were significantly higher in patients with RA compared to the control group. RA patients showed statistically significant higher in WBC and Ptt tests while they had lower RBCs, Hb, and RDW compared to controls. When compared to healthy individuals, we observed that patients with RF had greater levels of cytokines IL-1 $\beta$ , IL-2, IL-4, and IL-10. RF patients had higher levels of IL-1 $\beta$ , IL-2, IL-4, and IL-10 than did healthy people.

**Keywords:** Rheumatoid arthritis, Hematological parameters, Immunological parameters.

### 1. Introduction

The primary cause of persistent arthritis and bone loss is rheumatoid arthritis, an inflammatory disease that is thought to be genetically complicated [1]. Rheumatoid arthritis [RA] is caused by a number of factors, including immunological, environmental, and genetic factors [2], the main clinical manifestation of RA is Inflammatory polyarthritis a chronic inflammatory disease that mostly disturbs the small joints of the hands and feet [3]. The many cellular and molecular mechanisms that are involved in the pathophysiology of RA eventually result in joint inflammation and destruction [4]. Rheumatoid factor [RF] production, synovial inflammation, cartilage and bone abnormalities, hyperplasia, and systemic features such as pulmonary, skin, skeletal, and psychological problems are the hallmarks of RA [5].

RA is one type of autoimmune disease that affects the synovial joints as a result of responses to certain pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and IL-17, in addition to T and B cells [6]. The level of certain cytokines in the bloodstream has been observed in many inflammatory conditions including RA and the diagnosis and severity of the disease is determined by knowing the levels of concentration of these cytokines [7]. A previous study showed a significant increase in the levels of some pro-inflammatory cytokines [TNF- $\alpha$  and INF- $\gamma$ ] in autoimmune diseases [8].

B cells, T cells, and other immune effector cells show a central role in releasing pro-inflammatory cytokines that activate fibroblast-like synovial cells and contribute to bone and cartilage lesions. The development of synovitis is facilitated by innate immune cells like mast cells, neutrophils, and macrophages, which release proinflammatory cytokines namely IL-1, and IL-6, and inflammatory mediators like prostanoids, nitrogen intermediates, and free oxygen radicals [9]. The study aimed to assess the changes in some hematological and immunological parameters in patients with RA disease.

## 2. Methods Section

This study was completed at Baquba Teaching Hospital from August 2023 to May 2024 to evaluate the changes in WBC, Hb, RBC, RDW, and Ptt and also to assay the immunological parameters [IL-1 $\beta$ , IL-2, IL-4, and IL-10] in patients with Rheumatoid Arthritis. Every individual had a total of 9 milliliters of blood drawn using a disposable syringe. Blood samples were dispensed into 2mL in an EDTA tube to perform a complete blood count [CBC] test. The test was carried out by a fully automated quantitative Samsung instrument. The erythrocyte sedimentation rate [ESR] test was performed on another 2 mL, and the remaining 5 ml were centrifuged to separate the serum and kept at -20°C until it was needed for the RF, CRP, and cytokine tests.

RF test and CRP test were measured by agglutination test. ESR was measured in accord with the International Committee for Standardization in Hematology's [ICSH] standard Westergren technique approach [10]. IL-1, IL-2, IL-4, IL-10, and IL-17 levels were detected by the sandwich ELISA technique made by Sunlong Biotech company [11].

Data Analysis: the results were expressed by SPSS statistical analysis as mean  $\pm$  SE or percentage [%] of case frequency. Data were analyzed for assessments using one-way analysis of variance [ANOVA], Fisher's test, or t-test. Statistical analysis was conducted using StatView 5.0, with differences considered significant at  $p < 0.05$ .

## 3. Results and Discussion Section

This study involved 90 samples taken from patients infected by RA disease and healthy control. The samples from patients infected by RA disease include 60 samples [66.6 %] and samples from control groups include 30 samples [33.3%]. Distribution of the studied groups according to their gender showed that the majority of RA patients were females [46.6%] compared with males was highly significant compared with other gender groups [ $P < 0.001$ ] as shown in Table 1.

**Table 1:** Distribution of study groups

Study groups		RF		P. value
		N	%	
Patient	Female	42	46.6	< 0.001
	Male	18	20	
Control	Female	17	18.8	0.041
	Male	13	14.4	
Total		90	100	

The incidence of RA among our studied females was 70%. This study agreed with a previous study in Iraq showing that common RA patients were females compared with males 79.7% and 75.7%, respectively [12,13]. Women are more susceptible to developing RA than males because of the hormonal variations between the genders and the ensuing consequences these variances have on the immune system. These differences cause women to activate more T-helper cells, which play a pro-inflammatory role and may accelerate the onset of autoimmunity [14].

The levels of RF, ESR, and CRP test were higher significantly in patients with RA [100%, 90%, and 76%, respectively] and compared to the control [0%,10%, and 24%, respectively] as shown in Table 2.

**Table 2:** The levels of RF, CRP, and ESR in patients' groups

Study groups	Patients [ N / 60]				P. value
	positive		negative		
RF test	60	100 %	0	0 %	<0.001
CRP test	54	90 %	6	10 %	<0.001
ESR test [mm/hour]	46	76 %	14	24 %	<0.001

RA patients demonstrated statistically significantly greater WBC and PTT compared to controls but reduced RBCs, Hb, and RDW as shown in Table 3.

**Table 3:** Complete blood count in patients and in the control group.

Parameter	Patients [Mean ± S.E]	Control [Mean ± S.E]	P. value
WBC [ $10^9$ /L]	10.21±2.11	7.23±1.31	0.068
Hb [g/dL]	11.31±1.42	12.9±2.52	0.041
RBC[ $10^{12}$ /L]	3.89±1.11	5.62±1.61	0.061
RDW %	12.2±3.21	13.7±3.02	0.036
Ptt [ $10^9$ /L]	391.2±36.3	336.8±28.9	0.071

Research demonstrated that platelets play a crucial part in the inflammatory response [15]. Regarding the RBC characteristics, compared to healthy controls, RA patients showed statistically significant increases in RDW% and decreases in RBC count, Hb level, and Hb/RDW% ratio. Previous studies found that the RDW test in RA patients was a higher significant compared to controls [16,17].

According to the previous study, patients with RA had a larger percentage of RDW than those with osteoarthritis [18]. The previous study showed that RA patients had significantly lower RBCs and higher platelet counts than the control group [19].

The results showed that there was a significant difference [ $P < 0.001$ ] in IL-1 $\beta$  levels, IL-4, and IL-10 between RA patients [76.21 $\pm$ 11.2 pg/ml, 58.02 $\pm$ 19.1 pg/ml and 26.01 $\pm$ 10.1 pg/ml] and control groups [28.02 $\pm$  8.12 pg/ml, 23.1 $\pm$ 5.21 pg/ml and 11.2 $\pm$ 2.1 pg/ml], respectively. The results showed that there was no significant difference [ $P > 0.05$ ] in IL-2 levels between RA patients [21.11 $\pm$ 0.21 pg/ml] and control groups [19.7 $\pm$ 0.15 pg/ml] patients, respectively], as demonstrated in table 4.

**Table 4:** Serum levels IL-1 $\beta$ , IL-2, IL-4 and IL-10 among studied groups

Parameter	Patients [Mean $\pm$ S.E]	Control [Mean $\pm$ S.E]	P. value
IL-1 $\beta$	76.21 $\pm$ 11.2 pg/ml	28.02 $\pm$ 8.12 pg/ml	0.001
IL-2	21.11 $\pm$ 0.21 pg/ml	19.7 $\pm$ 0.15 pg/ml	0.0381
IL-4	58.02 $\pm$ 19.1 pg/ml	23.1 $\pm$ 5.21 pg/ml	0.001
IL-10	26.01 $\pm$ 10.1 pg/ml	11.2 $\pm$ 2.1 pg/ml	0.001

IL-1 and IL-6 are proinflammatory cytokines that are important in the pathophysiology of RA [20]. IL-1 has a main role in RA [21]. The local production of TNF and IL-1 in the inflammatory synovial joint has a role in the etiology of RA, either directly or indirectly [RA] [22]. TNF- $\alpha$  and IL-1 cytokines, which are produced in huge quantities by mast cells, are essential for ongoing inflammation. When autoantibodies and complement fragments bind to mast cells, they may get the signal and produce TNF- $\alpha$  and IL-1, which can lead to synovial pathology and erosive arthritis [23].

A recent study examining the role of IL-2 in immunomodulation has shown that IL-2 can reduce autoantibody-induced autoimmunity [24]. Most previous studies have agreed that IL-2 is a pro-inflammatory cytokine and that its production exacerbates Th-1-mediated disease states such as RA [25]. Previous research results on human arthritis patients and animal models have shown that some Th-1 cytokines such as IL-2 and IFN- $\gamma$  can be pro-inflammatory, while Th-2 cytokines such as IL-4 and IL-10 can protect against arthritis [26]. Previous studies have shown the important role of variation in the levels of some immune cytokines such as IL-1 $\beta$  and IL-10 in the pathogenesis of rheumatoid arthritis and the level and severity of the disease [27], as a previous study showed that the level of IL-4 in patients with early RA was much higher than its level in the control group [28].

#### 4. Conclusion

This study presented that most of the RA patients were female, with a significant difference between female and male patients. Values of RF, ESR, and CRP tests in this study were significantly higher in RA patients. The present study also showed a statistically significant increase in WBC and PTT but a decrease in RBCs, Hb, and RDW in the patients compared with the control group. Levels of IL-1 $\beta$ , IL-2, IL-4, and IL-10 in RF patients were higher than in healthy individuals.

## Abbreviations

Erythrocyte precipitation rate	ESR
C-reactive protein	CRP
Complete blood count	CBC
White blood cell	WBC
Red blood cell	RBC
Hemoglobin	HB
A red cell distribution width	RDW
Platelets	Ptt

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