

## Role of tumor necrosis factor alpha (TNF- $\alpha$ ) and interferon $\gamma$ (IFN- $\gamma$ ) in rheumatoid arthritis iraqi patients

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### Abstract :

Rheumatoid arthritis (RA) is an autoimmune chronic inflammatory illness that affects the whole body and is characterized by non-articular involvement and inflammatory arthritis. In many instances, that is produced by the interplay between genes and environmental factors. This research was designed to determine the amount of cytokines that cause rheumatoid arthritis, such as TNF-alpha and IFN-  $\gamma$  in patients with rheumatoid arthritis in Al-Anbar governorate / Iraq and compare their results with healthy people who do not have the disease.

Results have shown that there is a definite and significant difference between the TNF-alpha values for the two groups studied. The results shown that the group of rheumatoid arthritis patients had a significant increase in the values of this variable to 44.99 pg/mL, while the values of this variable were within normal values (23.83 pg/mL). The results revealed that there was a significant increased in the level of IFN-  $\gamma$  in the group of patients with rheumatoid arthritis compared to people without rheumatoid arthritis (24.03 vs. 5.28) pg/mL.

**Keywords:** Cytokines, Rheumatoid arthritis, Tumor necrosis factor alpha (TNF- $\alpha$ ) and Interferon  $\gamma$  (IFN- $\gamma$ ).

### دور عامل نخر الورم ألفا (TNF- $\alpha$ ) والإنترفيرون جاما (IFN- $\gamma$ )

#### في مرضى التهاب المفاصل الروماتويدي العراقيين

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#### مستخلص:

التهاب المفاصل الروماتويدي (RA) هو مرض التهابي مزمن مناعي ذاتي يصيب الجسم كله ويتميز بإصابة غير مفصلية والتهاب المفاصل. وفي كثير من الحالات، ينتج ذلك عن التفاعل بين الجينات والعوامل البيئية. وقد صمم هذا البحث لتحديد كمية السيتوكينات المسببة لالتهاب المفاصل الروماتويدي، مثل عامل نخر الورم ألفا (TNF- $\alpha$ ) والإنترفيرون جاما (IFN- $\gamma$ ) في مرضى التهاب المفاصل الروماتويدي في محافظة الأنبار / العراق ومقارنة نتائجهم مع الأشخاص الأصحاء الذين لا يعانون من المرض.

أظهرت النتائج أن هناك فرقاً واضحاً وهاماً بين قيم عامل نخر الورم ألفا (TNF- $\alpha$ ) للمجموعتين المدروستين. أظهرت النتائج أن مجموعة مرضى التهاب المفاصل الروماتويدي كان لديها زيادة كبيرة في قيم هذا المتغير إلى 44.99 بيكوغرام / مل، في حين كانت قيم هذا المتغير ضمن القيم الطبيعية (23.83 بيكوغرام / مل). كشفت النتائج عن وجود زيادة كبيرة في مستوى الإنترفيرون جاما (IFN- $\gamma$ ) في مجموعة المرضى الذين يعانون من التهاب المفاصل الروماتويدي مقارنة بالأشخاص غير المصابين بالتهاب المفاصل الروماتويدي (24.03 مقابل 5.28) بيكوغرام / مل.

**الكلمات المفتاحية:** السيتوكينات، التهاب المفاصل الروماتويدي، عامل نخر الورم ألفا والإنترفيرون جاما.

## 1. INTRODUCTION:

Rheumatoid arthritis (RA) is a kind of autoimmune illness that affects the whole body and is characterized by non-articular involvement and inflammatory arthritis. In many instances, it is a chronic inflammatory illness that is produced by the interplay between genes and environmental factors. The synovial joints are the primary target of this condition. The condition normally begins in the smaller joints of the periphery, is typically symmetric, and, if it is not treated, eventually spreads to the joints of the proximal extremities<sup>[1]</sup>. Over time, joint degradation, including bone erosion and cartilage loss, may be caused by an infection. For RA to be considered early, symptoms must have persisted for less than six months; for RA to be considered established, symptoms must have persisted for more than six months. Chronic inflammatory disorder (RA) is a disease that worsens with time and increases the risk of death if not treated<sup>[2]</sup>.

In the early stages of rheumatoid arthritis, the diagnosis of this condition might be difficult since no laboratory test can definitively identify the pres-

ence of the disease. A complete clinical approach is essential for diagnosing and preventing joint injury that might be disabling. Patients suffering from rheumatoid arthritis need treatment that includes both pharmacological and non-pharmacological therapy to be effectively treated. The early treatment of rheumatic illness with disease-modifying antirheumatic medications is now considered the standard of therapy. Many patients, despite receiving therapy, eventually become disabled and have severe morbidity during their illness<sup>[3]</sup>.

This study aimed to determine the amount of cytokines that cause rheumatoid arthritis, such as TNF-alpha and IFN- $\gamma$  in patients with rheumatoid arthritis in Al-Anbar governorate / Iraq and compare their results with healthy people who do not have the disease.

## 2. MATERIALS AND METHODS

In this study, two groups were chosen to study the effect of some laboratory parameters, such as cytokines that cause rheumatoid inflammation for patients visiting health institutions in Al-Anbar Governorate /Iraq for the period from 2/1/2023 to 30/8/2023,

who had symptoms of rheumatoid arthritis, while comparing the results of their laboratory tests with the results of healthy people for comparison.

The first group (control group) consisted of 50 healthy people without rheumatoid arthritis. The second group (patient group) consisted of 100 patients with rheumatoid arthritis. Blood samples were taken from the two study groups using sterile wine syringes and sent to the laboratory to conduct the necessary laboratory tests. Information was taken from the patient, which included the patient's age, gender, weight, height, and medical history. The ages of the patients ranged from 28 to 70 years for both study groups and for both sexes.

### 2.1. Complete blood count (CBC)

This test was conducted to calculate the total number of red blood cells (RBCs) and the total number of white blood cells (WBCs) in the blood samples of the two study groups (the rheumatoid arthritis patients' group and the healthy people' group). Blood samples of 3-5 mL were taken from the people under study and sent to the laboratory to count the number of red blood cells and the number of white blood cel-

ls using a blood cell counting device produced by the Indian company Indiamart.

### 2.2. Estimation of TNF- $\alpha$ in blood sample

Before adding samples and standards to the wells of a microplate, a monoclonal antibody specific to TNF- $\alpha$  is pre-coated on it. Any TNF- $\alpha$  that is present is bound with the immobilised antibody. The wells should be prepared by adding any unbound substances and an enzyme-linked polyclonal antibody specific for TNF- $\alpha$ . After that, wash the wells to remove any unbound antibody reagent. Then, add a substance solution. The colour will change according to the amount of TNF- $\alpha$  in the initial step. Once the colour change has stopped, measure the intensity of the sample's colour.

### 2.3. Estimation of IFN- $\gamma$ in blood sample

To coat microwells, an anti-human IFN- $\gamma$  monoclonal antibody is adsorbed. When antibodies are adsorbed to microwells, human IFN- $\gamma$  in the sample or standard attaches to them. Then, a biotin-conjugated monoclonal anti-human IFN- $\gamma$  antibody binds to the human IFN- $\gamma$  that was caught by

the first antibody. Streptavidin-HRP attaches to the anti-human IFN- $\gamma$  biotin conjugated antibody.

Substrate solution reactive with HRP is added to the wells after the incubation period, and the wash step removes the unbound biotin conjugated anti human IFN- $\gamma$  and Streptavidin-HRP. The quantity of soluble human IFN- $\gamma$  in the sample determines the formation of the coloured product. To stop the process, we add acid and then measure the absorbance at 450

nm. Seven standard dilutions of human IFN- $\gamma$  and the concentration of human IFN- $\gamma$  in the sample are used to create a standard curve.

#### 2.4. Statistics Analysis

Use the statistical analysis program (SPSS) Ver. 22.0 to analyze the results and find significant differences between the tested parameters for the two groups studied and the relationship between the variables.

### 3. RESULTS AND DISCUSSION

**Table 1:** The clinical characteristics of the included participant

Variables	Group	Mean	N	Std. Deviation	
<b>Age</b>	Control	47.6000	50	9.71849	0.001
	Rheumatoid arthritis	58.3300	100	9.48157	
	Total	54.7533	150	10.79581	
<b>BMI</b>	Control	27.2500	50	3.28697	0.001
	Rheumatoid arthritis	38.6045	100	3.79172	
	Total	34.8197	150	6.47672	
<b>ESR</b>	Control	15.3420	50	2.57183	0.001
	Rheumatoid arthritis	24.2440	100	7.19716	
	Total	21.2767	150	7.37023	
<b>RBC</b>	Control	5.0576	50	0.51324	0.001
	Rheumatoid arthritis	3.7355	100	0.29990	
	Total	4.1762	150	0.73309	
<b>WBC</b>	Control	6.8030	50	0.40386	0.001
	Rheumatoid arthritis	7.7810	100	0.43755	
	Total	7.4550	150	0.62835	
<b>NTF</b>	Control	23.8380	50	3.48254	0.001
	Rheumatoid arthritis	44.9930	100	11.74432	
	Total	37.9413	150	13.99114	
<b>IFN</b>	Control	5.2842	50	1.48727	0.001
	Rheumatoid arthritis	24.0320	100	7.07615	
	Total	17.7827	150	10.61261	

### 3.1 The Age of Patients of Groups Studied

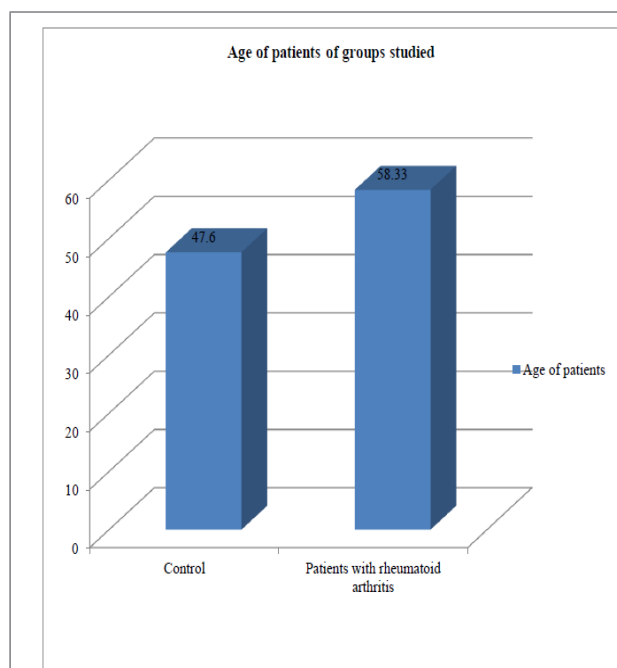
The two research groups in this study had ages ranging from 28 to 70 years old. Details are shown in Table 1. The results of Table 1 indicated that the average age of patients with rheumatoid arthritis was 58.33 years, their ages ranged from 32 to 70 years, while the average age of healthy people was 47.60 years, and their ages ranged from 28 to 65 years respectively. The results of this table were consistent with the results of the study conducted by researcher Kato *et al.* <sup>[4]</sup>, which confirmed that the average age of patients with rheumatoid arthritis was 55.8 years during the year 2002-2003, 57.0 years during the year 2007-2008, and 59.9 years during the year 2012-2013.

In a study conducted in 2023 by Yu and others <sup>[5]</sup>, it was shown that the risk of developing rheumatoid arthritis is greatly affected by the patient's age. The study confirmed that early detection of the disease and treatment in the early stages impact controlling the disease and reducing its harms. It also showed that health education and

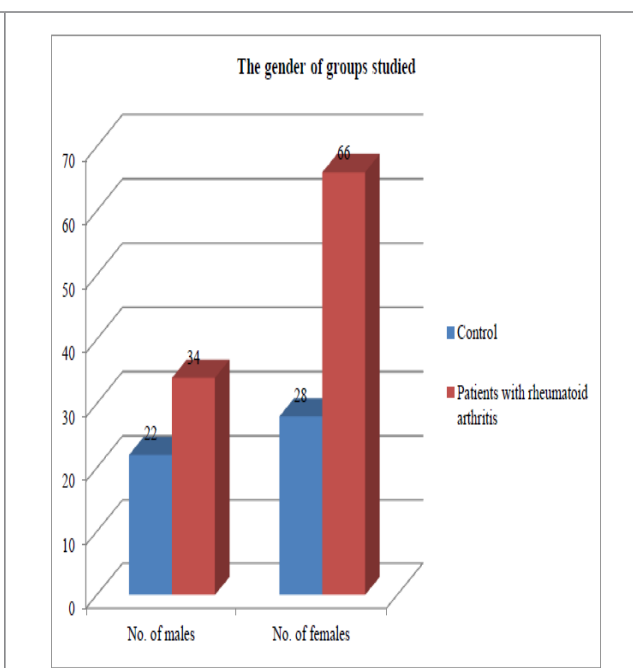
health programs that aim to control the disease in middle-aged patients has a major role in treating the disease. According to Bullock *et al.* <sup>[6]</sup>, risk factors for developing rheumatoid arthritis are age, gender, heredity, and environmental stressors such as smoking and air pollutants.

### 3.2. The Gender of Groups Studied

The results of Table 1 showed that women are more susceptible to rheumatoid arthritis, as 66 women out of a total of 100 cases were recorded, while the number of infected males was 34 males. Vollenhaven <sup>[7]</sup> showed that females are three times more susceptible to rheumatoid arthritis than males. Also, Maranini *et al.* <sup>[8]</sup> indicated that rheumatoid arthritis is a chronic inflammatory disease affecting females with a ratio of 3:1 (female/male). The role of gender in susceptibility of females to rheumatoid arthritis may be due to hormonal factors and the effect of sexual dysmorphism <sup>[9,10]</sup>. While Alpizar-Rodriguez <sup>[11]</sup> and Karlson *et al.* <sup>[12]</sup> indicated that pregnancy and breastfeeding have been linked with a decreased risk of women with rheumatoid arthritis.



**Figure 1:** Age of patients of groups studied



**Figure 2:** The gender of the groups studied

### 3.3. BMI of Groups Studied

The results of Table 1 indicated that the two study groups (the rheumatoid arthritis patient group and the control group) differed in average BMI values, as the patient group was significantly superior in increasing BMI values, recording 38.60 kg/m<sup>2</sup> compared to 27.25 kg/m<sup>2</sup> for the control group.

It is clear that the values of the patient group indicate that patients with rheumatoid arthritis suffer from obesity, while the values of the control group were within normal ranges. In a study conducted by Abuhelwa *et al.* <sup>[13]</sup>, out of a total of 5428 people, 32.8% were

above normal weight (overweight), 30.4% were obese, and 33.9% had a normal BMI. While, Qin *et al.* <sup>[14]</sup> showed that the elevation in BMI was associated with the increasing risk of rheumatoid arthritis development.

### 3.4. ESR Values of Groups Studied

The results of Table 1 showed that the two tested groups differed significantly in the ESR values. The group of patients with rheumatoid arthritis achieved a significant increase in the ESR value, reaching 24.24, while the control group recorded 15.34, respectively. The elevation of ESR value in patients with rheumatoid arthritis may indicate the presence of an inflamma-

tory process in the body. In a study by Jassim *et al.* [15], it was confirmed that there was a significant increase in

ESR values for the rheumatoid arthritis patient group in comparison to the of healthy people.

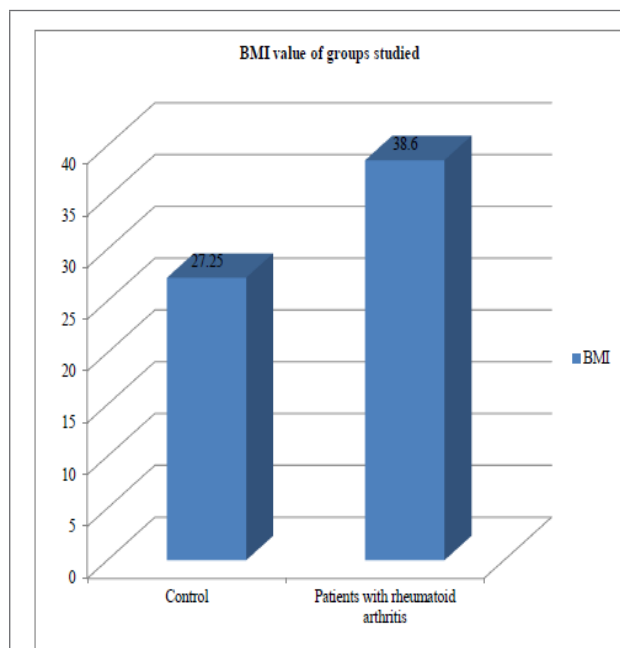


Figure 3: BMI value of groups studied

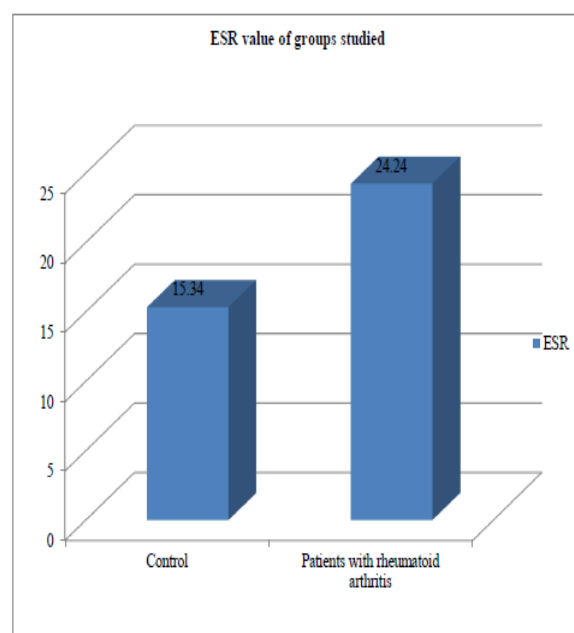


Figure 4: ESR value of groups studied

### 3.5. RBC Count of Groups Studied

The data in Table1 revealed that there was a significant decrease in the average red blood cell count values for the rheumatoid arthritis patient group in comparison to the of healthy people. The patients group recorded  $3.73 \times 10^6$  cells/mL, while the control group recorded  $5.05 \times 10^6$  cells/mL.

In rheumatoid arthritis, the immune response leads to inflammation of the joints and various tissues, and this chronic inflammation leads to a reduction in the production of red blood cells

in the bone marrow. Chronic rheumatoid inflammation is associated with types of anemia, especially iron deficiency anemia [16,17].

Xue *et al.* [18] showed that patients with active rheumatoid arthritis have a significant low level of RBC counts and haemoglobin contents as compared with inactive rheumatoid arthritis, and they concluded that RBC and PLT were significantly linked with rheumatoid arthritis disease activity and may be these parameters used to detective active rheumatoid arthritis from inac-

tive RA. It was found that inflammatory cytokines may decreased the count of RBC, and it has been concluded that parameters related with RBC such as RBC count and hemoglobin levels can be utilized as markers to anticipate the degree of a number of illnesses, such as rheumatoid arthritis [19].

### 3.6. WBC Count of Groups Studied

The results of Table1 revealed that there was a significant difference in the white blood cell count rate for the two tested groups. The results showed that the group of patients with rheu-

matoid arthritis had a significant elevation in the white blood cell count rate to  $7.78 \times 10^3$  cell /  $\mu$ L, while the group of healthy people recorded  $6.80 \times 10^3$  cell/ $\mu$ L.

Farouk *et al.* [20] indicated that WBC count in patients with rheumatoid arthritis significantly increased ( $7.13 \times 10^9$ /L) as compared with healthy persons ( $6.97 \times 10^9$ /L). They pointed out that WBC and RBC parameters are significantly associated with disease activity in RA patients, and it may be utilized as indicators in activity and inflammation of patients with RA.

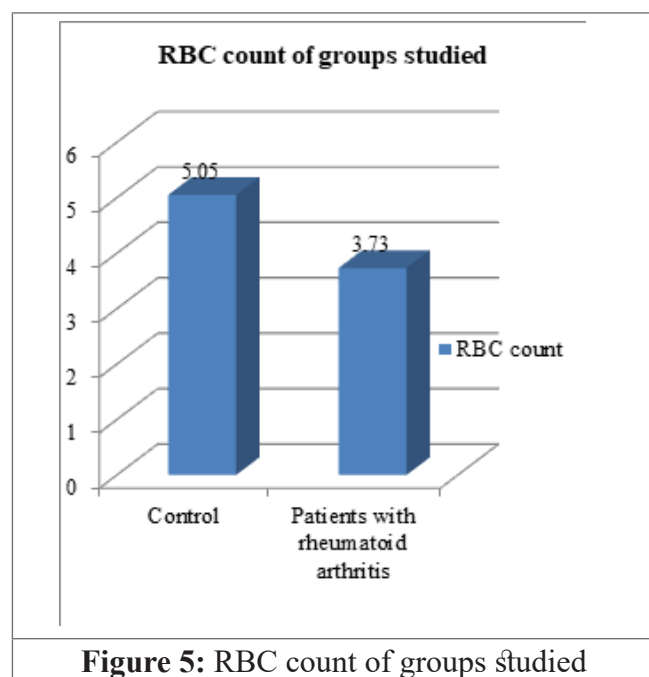


Figure 5: RBC count of groups studied

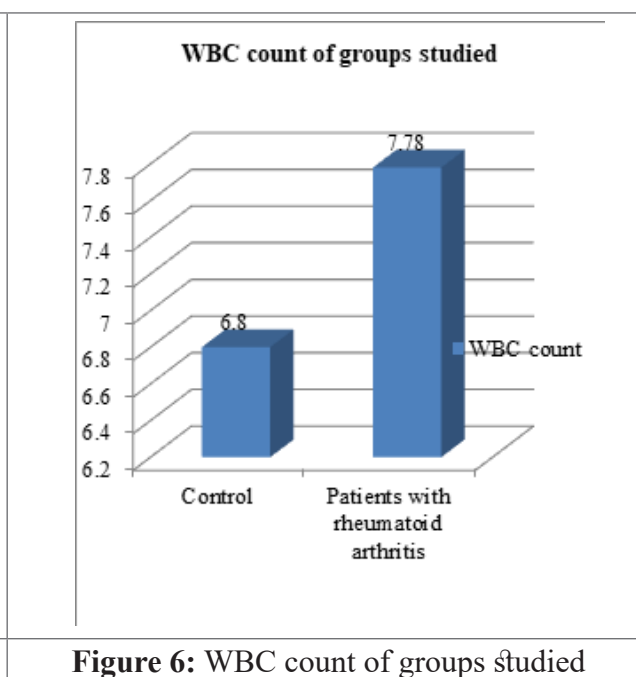


Figure 6: WBC count of groups studied

### 3.7. TNF-alpha Level of Groups Studied

The results of Table1 showed that

there is a clear significant difference between the TNF-alpha values for the two groups studied. The results showed

that the group of rheumatoid arthritis patients had a significant increase in the values of this variable to 44.99 pg/mL, while the values of this variable were within normal values (23.83 pg/mL). The cytokine tumor necrosis factor alpha (TNF- $\alpha$ ) affects a variety of cell types in pleiotropic ways. It has a significant role in the pathophysiology of some inflammatory and autoimmune disorders and is expressed as a primary regulator of inflammatory responses [21]. Chronic inflammation is linked to inappropriate or excessive TNF- $\alpha$  signaling activation, which can result in pathological consequences such autoimmune disorders. The cytokine TNF-alpha acts in rheumatoid arthritis by activating synovial fibroblasts, which leads to excessive production of cathepsins and MMP and leads to the breakdown of collagen and proteogly-

cans, destroying cartilage and bone and joint erosion. Osteocytes in rheumatoid arthritis stimulate synovial hyperplasia and angiogenesis [22].

TNF $\alpha$  is thus considered a master regulator of pro-inflammatory cytokine production. TNF $\alpha$  increases adipose signal transduction mediators such as prostaglandins and platelet-activating factor. While, Thilagar *et al.* [23] indicated that TNF-alpha play an important role in chronic inflammatory diseases. Delima *et al.* [24] showed that TNF- $\alpha$  is active at the site of inflammation and in the circulation and is responsible for the changes that occur in systemic inflammation. The results of Table 2 revealed that there is a positive significant correlation between TNF-alpha and groups studied which recorded 0.715 at a level of 0.01.

**Table 2:** Correlation between TNF-alpha and groups studied

		Group	TNFalpha
Group	Pearson Correlation	1	.715**
	Sig.(2-tailed)		.000
	N	150	150
TNFalpha	Pearson Correlation	.715**	1
	Sig.(2-tailed)	.000	
	N	150	150
** Correlation is significant at the 0.01 level (2-tailed)			

### 3.8. IFN-Gamma Level of Groups Studied

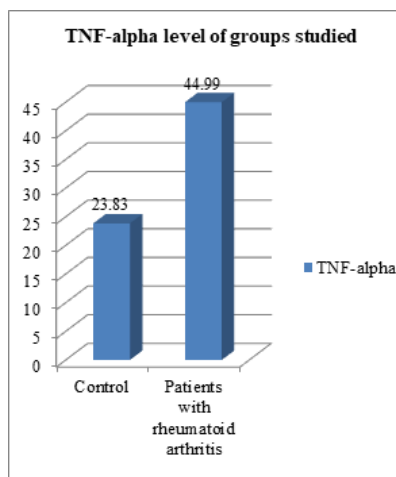
Table 2 revealed that there was a significant increase in the level of IFN-gamma in the group of patients with rheumatoid arthritis compared to people without rheumatoid arthritis (24.03 vs. 5.28) pg/mL.

Interferon  $\gamma$  (IFN- $\gamma$ ) induces an inflammatory response and apoptosis. Rheumatoid arthritis (RA) is known to be a systemic inflammatory disease

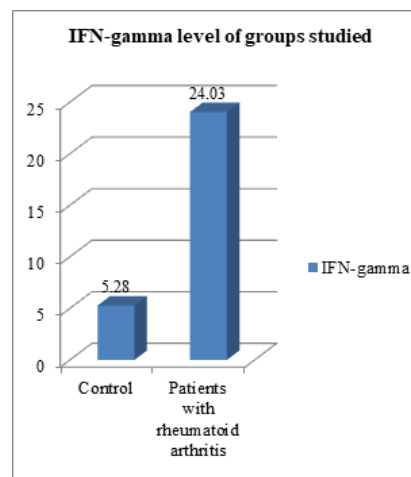
associated with elevated levels of inflammatory mediators, including tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) and helper T cells. Th 17, downregulating apoptosis of inflammatory cells. It was hypothesized that IFN- $\gamma$  would reduce inflammatory cell death in vitro and that loss of IFN- $\gamma$  would exacerbate inflammation in vivo [25]. While, Lee *et al.* [26] showed that the level of IFN-gamma is significantly elevated at the beginning of the disease in patients with rheumatoid arthritis.

**Table 3:** Correlation between IFN-gamma and groups studied

		Group	IFNgamma
Group	Pearson Correlation	1	.836**
	Sig.(2-tailed)		.000
	N	150	150
IFNgamma	Pearson Correlation	.836**	1
	Sig.(2-tailed)	.000	
	N	150	150
** Correlation is significant at the 0.01 level(2-tailed)			



**Figure 7:** TNF- $\alpha$  level of groups studied



**Figure 8:** IFN-gamma level of groups studied

#### 4. CONCLUSIONS

##### AND RECOMMENDATION

In this study, the two research groups had ages ranging from 28 to 70 years old. The results indicated that the average age of patients with rheumatoid arthritis was 58.33 years, their ages ranged from 32 to 70 years, while the average age of healthy people was 47.60 years, and their ages ranged from 28 to 65 years respectively. The results showed that women are more susceptible to rheumatoid arthritis, as 66 women out of a total of 100 cases were recorded, while the number of infected males was 34 males. The results indicated that the two study groups (the rheumatoid arthritis patient group and the control group) differed in average BMI values, as the patient group was significantly superior in increasing BMI values, recording 38.60 kg/m<sup>2</sup> compared to 27.25 kg/m<sup>2</sup> for the control group. The results showed that the two tested groups differed significantly in the ESR values. The group of patients with rheumatoid arthritis achieved a significant increase in the ESR value, reaching 24.24, while the control group recorded 15.34, respec-

tively.

The data revealed that there was a significant decrease in the average red blood cell count values for the rheumatoid arthritis patient group in comparison to the of healthy people. The patients group recorded  $3.73 \times 10^6$  cells/mL, while the control group recorded  $5.05 \times 10^6$  cells/mL. The results revealed that there was a significant difference in the white blood cell count rate for the two tested groups. The results showed that the group of patients with rheumatoid arthritis had a significant elevation in the white blood cell count rate to  $7.78 \times 10^3$  cell /  $\mu$ L, while the group of healthy people recorded  $6.80 \times 10^3$  cell/ $\mu$ L.

The results showed that there is a clear significant difference between the TNF-alpha values for the two groups studied. The results showed that the group of rheumatoid arthritis patients had a significant increase in the values of this variable to 44.99 pg/mL, while the values of this variable were within normal values (23.83 pg/mL).

The results revealed that there was a significant increase in the level of IFN-gamma in the group of patients

with rheumatoid arthritis compared to people without rheumatoid arthritis (24.03 vs. 5.28) pg/mL. Monitoring and reducing the weight of a person suffering from rheumatoid arthritis has a significant impact on reducing damage as well as controlling the disease.

#### 4.1 Recommendations

It is necessary to perform periodic laboratory tests, which include tests of the red blood cell count and white blood cell count, and monitor the changes from their normal values, which may indicate inflammatory diseases such as rheumatoid arthritis. High ESR levels may be an indication that the patient is suffering from rheumatoid arthritis. Procedure blood tests, which include inflammatory cytokines such as TNF- $\alpha$  and IFN- $\gamma$  and monitoring any changes in their values from normal values, is of great importance in diagnosing patients with rheumatoid arthritis.

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