

The Role Of Gamma-Interferon And Interleukin12 In The Pathogenesis Of Rheumatoid Arthritis Patients In Iraq

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ABSTRACT

Background: Objectives: To evaluate the significant importance of IFN- γ and IL-12 level in Rheumatoid Arthritis patients. **Material and Methods:** A serum sample was collected from two groups. The first group was patients with Rheumatoid Arthritis (RA), the Second group was healthy volunteers. serum of this sample used to detect IFN- γ and IL12 level was quantified by using ELISA. **Result:** The level of serum INF-gamma was significantly lower in patients with rheumatoid arthritis in comparison with control group, 54.01 (14.81) versus 62.55 (28.11), respectively ($p = 0.006$). The level of serum IL-12 was significantly lower in patients with rheumatoid arthritis in comparison with control group, 8.47 (2.44) versus 132.82 (56.30), respectively ($p < 0.001$).

Conclusion: On the basis of the current study, the level of serum INF-gamma was significantly lower in patients with rheumatoid arthritis it means have no significant effect on disease susceptibility of patients. This study has found that the level of serum IL-12 was significantly lower in patients with rheumatoid arthritis.

Key words: RA, IL12,IFN- γ .

Article Information

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INTRODUCTION

Rheumatoid arthritis(RA) is a systemic autoimmune disease characterized by inflammatory arthritis and extra-articular involvement. It is a chronic inflammatory disorder of unknown etiology that primarily involves synovial joints. It typically starts in small peripheral joints, is often symmetric, and progresses to involve proximal joints if left untreated(1).Joint inflammation over time leads to the destruction of the joint with cartilage and bone erosion. RA with a symptom duration of fewer than six months is defined as early RA, and when the symptoms have been present for more than six months, it is defined as established RA (1).There is no pathognomonic laboratory test for rheumatoid arthritis, which makes the diagnosis of this disease challenging. An astute and comprehensive clinical approach is required to make the

diagnosis and prevent debilitating joint damage (1).

The cytokine interferon-gamma (IFN- γ), which is the sole member of type II interferons, plays an important role in the innate and adaptive immune responses. Since it activates monocytes/macrophages and induces the expression of major histocompatibility complex (MHC) class II on the cells, several basic and clinical studies have hypothesized its contribution to the development and progression of rheumatoid arthritis (RA), an autoimmune disorder accompanied by the activation of monocytes/macrophages (2). Interferon-gamma, a cytokine highly related with the Th1 response, is an important regulator of IgG2a antibody formation, a subclass commonly linked with a pathogenic autoantibody response. As a result, IFN- has

been identified as a main candidate for autoimmune modulation(3). Interleukin -12 is a hetero dimeric and pleiotropic cytokine which was initially known as natural killer cell stimulatory factor (NKSF)(4).Interleukin-12 is involved in the pathogenesis of rheumatoid arthritis. It is generated by cells that transmit antigens to the immune system, such as monocytes, macrophages, and dendritic cells. It promotes IFN production, which is essential for the development of naive T cells into Th1 cells.IL-12 is currently regarded as an important cytokine in regards to the regulation of Th1/Th2 balance (5)(6).

MATERIALS AND METHODS.

This study included Iraqi people (as patients) with RA who visit Rheumatology department in Al-Sadr Medical City in Najaf. This study has been conducted on 50 RA patients which were diagnosed with Rheumatoid arthritis (according to rheumatologist physicians in accordance with ACR/EULAR 2010 Criteria and serological tests). They were 1 male and 49 females, and the patients age was 20-70 years. All patients were questioned by rheumatologist and researcher about name, age, gender, DM, HT, smoking, RA-family history and other questions as mentioned in questionnaire. Patient disease duration at that minimum one month and maximum 10 years and according to the data in rheumatologist question from RA patients researcher done the DAS-28-ESR for patients to classify RA patients depending on the DAS28-ESR to mild, moderate and sever . The blood sample was drawing and put in sterile gel tube, centrifuged for separation of serum after allowing to clot at the room temperature , after that serum sample was put in Eppendorf tube for each one patient and kept at -20 to -45 °C until used. In addition to that about 50 healthy volunteers were included as a control group.

The Assay of Human Interleukin 12 and gamma-Interferon (IFN- γ) done by Enzyme-Linked Immunosorbent Assay (ELISA) ,(Bioassay technology laboratory(BT LAB)/China). The plate has been pre-coated with human IFN- γ and IL-12 antibodies .IFN- γ and IL-12 present in the samples are added and binds to antibodies coated on the wells. And then biotinylated human IFN- γ and IL-22

Antibodies are added and binds to IFN- γ and IL-12 in the samples. Then Streptavidin-HRP is added and binds to the Biotinylated IFN- γ and IL-22 antibodies. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human IFN- γ and IL-12. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

RESULTS

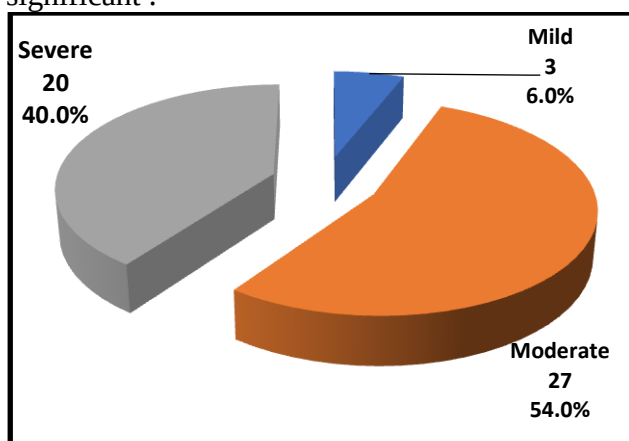
The Demographic characteristics of patients and control subjects enrolled in this study are shown in table 1. The man age of patients was 45.44 ± 11.39 years and it ranged from 20 to 70 years and that of control group was 33.53 ± 8.35 years and it ranged from 22 to 56 years; patients were therefore significantly older than control group ($p < 0.001$). There was no significant difference in gender distribution between patients and control groups ($p = 0.307$).The study included 50 patients with rheumatoid arthritis and the duration of disease was ranging from one month to 10 years with a mean of 3.58 ± 2.99 years. The disease severity was assessed according to DAS-28 scoring system which ranged from 2.57 to 5.59 and the mean was 4.65 ± 0.85 and patients were accordingly classified into 3 patients (6 %) with mild disease, 27 patients (54 %) with moderate disease and 20 cases (40 %) with severe disease shown in figure1.

Evaluation of cytokine levels in patients with rheumatoid arthritis compared to control group is shown in table 2. The level of serum INF-gamma was significantly lower in patients with rheumatoid arthritis in comparison with control group, 54.01 (14.81) versus 62.55 (28.11), respectively ($p = 0.006$) shown in figure 2. The level of serum IL-12 was significantly lower in patients with rheumatoid arthritis in comparison with control group, 8.47 (2.44) versus 132.82 (56.30), respectively ($p < 0.001$)shown in figure 3. Correlation of serum cytokine level to other characteristics is shown in table 3. Serum IFN-gamma showed significant negative correlation to age, CRP, RF and ESR and significant positive correlation to anti-CCP. Serum IL-12 showed significant negative correlation to age, CRP, RF and ESR.

Table 1: Demographic characteristics of patients and control subjects enrolled in this study.

Characteristic	Patients <i>n</i> = 50	Control <i>n</i> = 50	<i>p</i>
Age (years)			
Mean ±SD	45.44 ±11.39	33.53 ±8.35	<0.001 I ***
Range	20 -70	22 -56	
Gender			
Male, <i>n</i> (%)	1 (2.0 %)	3 (6.0 %)	0.307 Y NS
Female, <i>n</i> (%)	49 (98.0 %)	47 (93.0 %)	

n: number of cases; SD: standard deviation; I: independent sample t-test; Y: Yates correction test; ***: significant at $p \leq 0.001$; NS: not significant.

**Figure 1: Pie chart showing classification of patients with rheumatoid arthritis according to severity of disease based on DAS-28.****Table 2: Evaluation of cytokines levels in patients with rheumatoid arthritis compared to control group.**

Characteristic	Patients n = 50	Control n = 50	p
INF-γ			
Median (IQR)	54.01 (14.81)	62.55 (28.11)	0.006 M **
Range	34.33 - 318.11	24.57 - 344.31	

IL-12

Median (IQR)	8.47 (2.44)	132.82 (56.30)	< 0.001 M ***
Range	0.91 - 43.00	47.58 - 1376.78	

n: number of cases; M: Mann Whitney U test; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

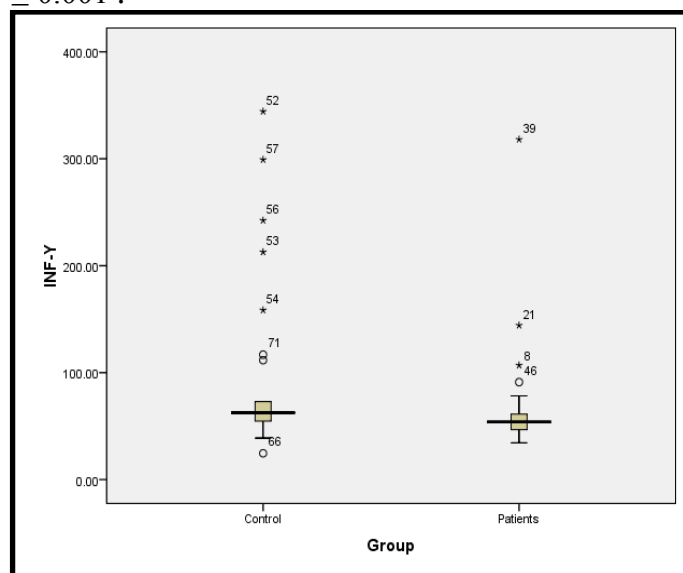
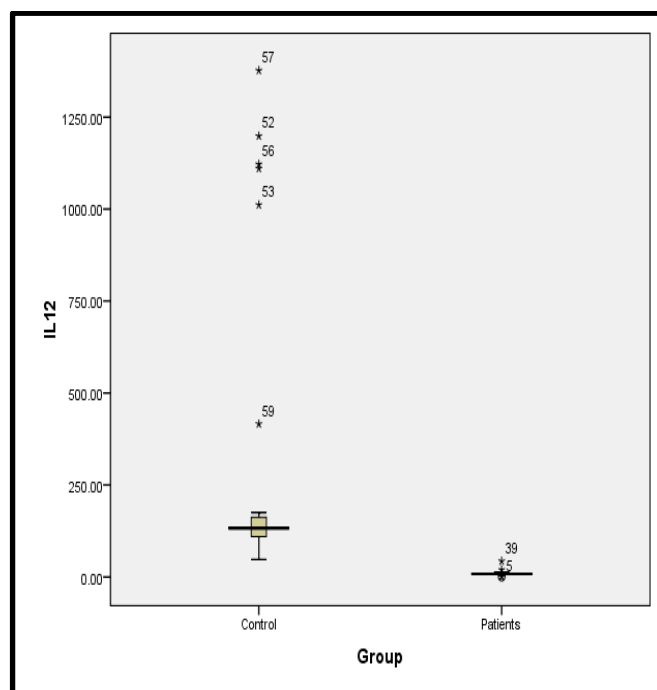
**Figure 2: Box plot showing comparison of median INF-gamma between control group and patients with rheumatoid arthritis.****Figure 3: Box plot showing comparison of median IL-12 between control group and patients with rheumatoid arthritis.**

Table 3: Correlation of serum cytokine level to other characteristics.

Characteristic	INF- γ		IL-12	
	r	p	r	p
Gender	-0.127	0.262	-0.144	0.203
Age	-0.251	0.025*	-0.414	< 0.001***
CRP	-0.125	0.267	-0.375	0.001***
RF	-0.132	0.244	-0.398	< 0.001***
ESR	-0.134	0.236	-0.461	< 0.001***
Anti-CCP	0.018*	0.903	0.118	0.413
DAS28	0.069	0.632	0.029	0.841

*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$

DISCUSSION

The present study agreement with Al-Rubaye et al. ;(2017) , Cope (2019)and Sura et al.,(2021). which demonstrated age at onset is usually between 30 – 70(7) , high incidence of RA among age groups 45- 75 years(8) and majority of RA patients btween40- 59(9), respectively.In regards of the mean age of patients, the present study shows that the mean age is 45.44 ± 11.39 years found in patients with RA. This result is compatible with Fathi et al. (2018) , that indicated the mean age in years was 49.1 ± 13 (10).The higher frequency of RA in females implies that female hormonal variables contribute to the disease's development. The peak of RA incidence occurs in the fifth decade of life, around the time of menopause in women. Oestrogen, for example, have been proposed to have a pro-inflammatory effect .

In this study the level of serum INF-gamma was significantly lower in patients with rheumatoid arthritis in comparison with control group, 54.01 (14.81) versus 62.55 (28.11),

respectively ($p = 0.006$). This result agreement with Olga M et al.,2022 That indicated to Intriguingly, RA patients had an almost lower concentration of IFN- γ compared to healthy control individuals(11).Another study done by Wei Zhong et al.,(2017)also consistent with present study that indicated to Following treatment, levels of IFN- γ significantly reduced in patients with RA(12).While the result disagree with Itzel Viridiana et al.,2019 and Voja Pavlovic et al.,(2013) that they indicated to serum level of IFN-y were increased in RA patients compared with healthy control(13)(14).

The present study found The level of serum IL-12 was significantly lower in patients with rheumatoid arthritis in comparison with control group, 8.47 (2.44) versus 132.82 (56.30), respectively ($p < 0.001$) and this result disagree with study done by El-Hakeim et al.,2020 in Egypt that indicated to that the serum level of IL-12 was significantly increased in patients compared to control group(15).While another study done by Paradowska-Gorycka et al.,2017 also inconsistent with our study that demonstrated the serum IL-12 was elevated in RA patients in compared with control group(6). In this study indicate to s erum IFN-gamma showed significant negative correlation to age, CRP, RF and ESR and significant positive correlation to anti-CCP.This result agreement with Voja Pavlovic *et al.*,(2013) that indicated to the mean INF γ serum levels showed significant negative correlations with a DAS28 score in patients with early RA. And patient age(14).The current findings are in line with results of Olga M et al.,2022 that showed a negative correlation of IFN- γ with CRP(11).

Also the result of study consistent with Tukaj S *et al.* 2010 who found that there is negative correlation between IFN-y with DAS28 score(16). Another study conducted by Itzelet *et al.*,2019 agreement with our result which found There is positive correlation between level of Anti-CCP and IFN-y(13).

For Interleukin 12 (IL12) the current study findings showed significant negative correlation between IL12 with age, CRP, RF and ESR. This partially agreement with El-Hakeim *et al.*,2020 regard by age and ESR that found there is no correlation between serum level of IL-12 and age and ESR(15).And

disagree with its result regard by RF and Anti-ccp which showed there is positive correlation between IL-12 and RF and Anti-ccp(15). Another study conducted by Paradowska-Gorycka *et al.*, 2017 partially consistent with our study that found there is negative correlation with ESR, RF and Anti-ccp(6). And disagree with our findings in part of C-reactive protein (CRP) which found there is positive correlation with IL-12 serum level(6). While the finding of another study conducted by Mokhtar *et al.*, 2018 disagree with our study result which indicated to there is significant positive correlation between IL-12 serum level and RF, CRP, ESR and Anti-ccp(17).

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CONCLUSION

On the basis of the current study, the level of serum INF-gamma was significantly lower in patients with rheumatoid arthritis it means have no significant effect on disease susceptibility of patients.

This study has found that the level of serum IL-12 was significantly lower in patients with rheumatoid arthritis.

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