

Estimation Of Serum Level Of TNF- A In Patient With Rheumatoid Arthritis In Thi-Qar Province

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Abstract

Background: Rheumatoid arthritis (RA) is a progressive autoimmune inflammatory disease affecting 1% of the population. **Aim and objective:** Study designed to determine the role of Tumor Necrosis Factor alpha (TNF- α) in a group of patients with rheumatoid arthritis for specific period of time and healthy controls. **Subjects and methods:** Blood samples were taken from 68 patients with rheumatoid arthritis Hospital in Thi-Qar province/Iraq and 20 healthy controls. All the information were taken from the patients and controls. An Enzyme-linked immunosorbent assay was used for estimation TNF- α . **Results:** The study showed that females are more susceptible to RA than males. Also the study showed common age group between (41-50) years of age was more than other groups. TNF- α showed a significant increase in RA patient compared to a healthy peoples. The mean of TN- α in patient was (41.6 \pm 14.1) while in control (5.16 \pm 1.58), in male patient and control mean was (45.9 \pm 13.8), (4.88 \pm 1.48) while in female patient and control mean was (41.2 \pm 13.7), (5.26 \pm 1.65) respectively. **Conclusions:** The study conclude that the levels of TNF- α play the important role in patients with RA, also TNF- α is the initiator of inflammation associated with the disease. The genetic factors may have a vital role to progression the RA.

Keyword: Rheumatoid arthritis, Autoimmune diseases, TNF- α and Cytokines.

I. Introduction

Rheumatoid arthritis(RA) is a systemic autoimmune characterized by extra-articular involvement and inflammatory arthritis, is a chronic inflammatory autoimmune disease that primarily affects joints (Calabresi et al.,2018). The various mechanisms that contribute to the disease's onset are not fully understood. It has been suggested that a variety of environmental variables, including smoking, occupational exposure, and atmospheric pollutants, can act as trigger stimuli for the onset of RA. It is a chronic inflammatory condition with no known cause that mostly affects synovial joints, If left untreated, it often begins in tiny peripheral joints, progresses to involve proximal joints, and is frequently symmetric, Joint deterioration caused by cartilage and bone loss over time as a result of joint inflammation, Early RA is described as having symptoms that have been present for less than six months, while established RA is defined as having symptoms that have been present for more than six months (Chauhan et al.,2021).An auto-immune disease that affects the joints, bones, tendons, and ligaments over time, rheumatoid arthritis is marked by inflammation(Verma et al., 2019). RA happens in any age, but the prevalence of rheumatoid arthritis is higher for those aged between 40 and 70 years. Chronic synovitis is a basic feature of this disease, which causes discomfort, stiffness and swelling, which is gradually accompanied by deterioration of cartilage, bone loss, resulting joint defects and systemic complications (Clapp et al., 2016). Cytokines are a class of signaling molecules with a wide range of applications in cellular communication, Their molecular weights range from 8 to 40,000 KD (Catalan-Dibene et al., 2018) which are released by many bodily cells, including white blood cells such as macrophages, fibroblasts, and lymphocytes, in response to a range of stimuli, These proteins aid in controlling the growth of immune effector cells, and some cytokines, which are produced by almost all cells to control and impact the immune response, have direct effector activities of their own(Takeuchi and Akira, 2010). Between the acts of pro-inflammatory cytokines like tumor necrosis factor alpha

(TNF), interleukin-1 (IL-1), and anti-inflammatory cytokines like tumor growth factor beta TGF, interleukin-10 (IL-10), cytokines function in a self-regulatory manner to preserve homeostasis. Patients with RA have cytokines in their serum, synovial fluid, and tissue. Each cytokine's release sets off a particular chain of events, from the activation of macrophages to the subsequent release of pro-inflammatory cytokines like tumor necrosis factor alpha (TNF) and interleukin-1 (IL-1), to the clonal proliferation of T-cells and osteoclast activation. These cells' presence along with specific cytokines causes the maturation and activation of osteoclasts, which in turn causes erosions and bone resorption (Firestein, 2003). Tumor necrosis factor alpha (TNF- α) is a proinflammatory cytokine (protein) including 19 components that communicate through 29 receptors. These ligands play a role in inflammatory, viral, and autoimmune processes in human illness while also regulating normal processes including immunological responses (Sushama et al., 2019). Cytotoxicity to tumor cell lines was one of the first functions to be discovered which led to its name, tumor necrosis factor (Grell and Scheurich, 2001). The role of TNF- α in rheumatoid arthritis (RA), TNF- α is mainly secreted by macrophage and Th1 cells. In RA, TNF- α activates synovial fibroblasts, The breakdown of collagen and proteoglycan follows, resulting in cartilage and bone destruction, as well as joint erosion. Osteoclasts in RA induce synovial hyperplasia (Jang, 2021). TNF- α has an important role in the pathogenesis of RA, since it is overexpressed in the synovial joints of patients (Farrugia and Baron, 2016). TNF- α plays a principal role in RA and is one of the main cytokine that results in inflammation during RA. It is produced mainly by activated macrophages but it can also be produced by monocytes, fibroblasts, mast cells and NK cells (Vasanthi et al., 2007).

II. Material and Method

A. Study Design

In this study a total number were 108 participants were divided into 68 patients with rheumatoid arthritis (RA), their age ranged from (20 to more than 85) and gender comprised of [6(8.8%) males, and female 62(91.2%)] who attending to Al-Nassiryia teaching hospital and private clinic were complained of rheumatoid arthritis diagnosed according to the revised criteria formulated by the American College of Rheumatology (ACR). This study was carried out during the period between February to June 2022. In addition 40 healthy individuals [10(25%) males, and female 30 (75%)] non RA and other disease as control group, their age ranged between (19-65). Healthy groups were collected from general population with negative history of major illness and have no abnormal physical findings during clinical examination by specialist and their routine laboratory investigations were within normal.

B. Methods

Five ml of blood were taken from RA patient and healthy groups, and placed in a clot-activator tube to collect serum. The collected serum was stored at -20°C after being centrifuged at 3000 rpm for 10 minutes. Serum TNF- α levels in two groups were evaluated by using the ELISA Kit of the provided company (Elabscience - USA). All patients and healthy individuals signed the consent form, as well the hospital's rheumatologist provided assistance in the patient selecting process.

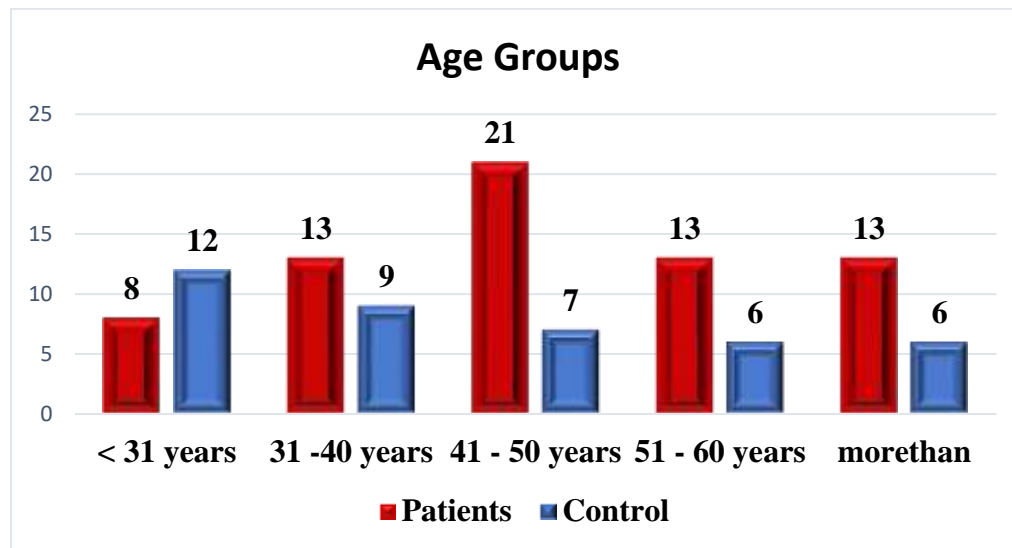
C. Statistical Analysis:

The experimental data were analyzed using the statistical program SPSS software kit (version 20.2) and Microsoft Excel 2016. Numerical data with normal distribution were described as mean and standard deviation. The lower level of accepted statistically significant difference is below or equal to 0.05 and the high level of accepted statistically significant difference is below or equal to 0.001. It was determined at ($P \leq 0.05$). Student's t-test and one way ANOVA.

III. Result and Discussions

A. Distribution of RA patient according age:

Some of the sociodemographic characteristics of 68 patients of rheumatoid arthritis and 40 healthy controls involved in this study. The age of the studied group ranged from (20 to more than 80) years with mean (48.6 ± 14.5) according to the age in rheumatoid arthritis patients are shown in (Figure-1). The results showed that the prevalence of the disease is more common in range age group (41-50) years in percentage (30.88%) and the range less than 30 was less percentage (11.76%). Thus, there were significant difference in distribution of RA patient according age (p.value < 0.001).



CalX ² = 6.412 TalX ² = 9.49 DF= 4 p. value 0.014 significant	
Patients Age Mean = 48.6 ± 14.5 (20-85 years)	0.003 Significant
Control Age Mean = 39.6 ± 14.5 (19-65 years)	

Figure 1. Distribution of RA patient according age

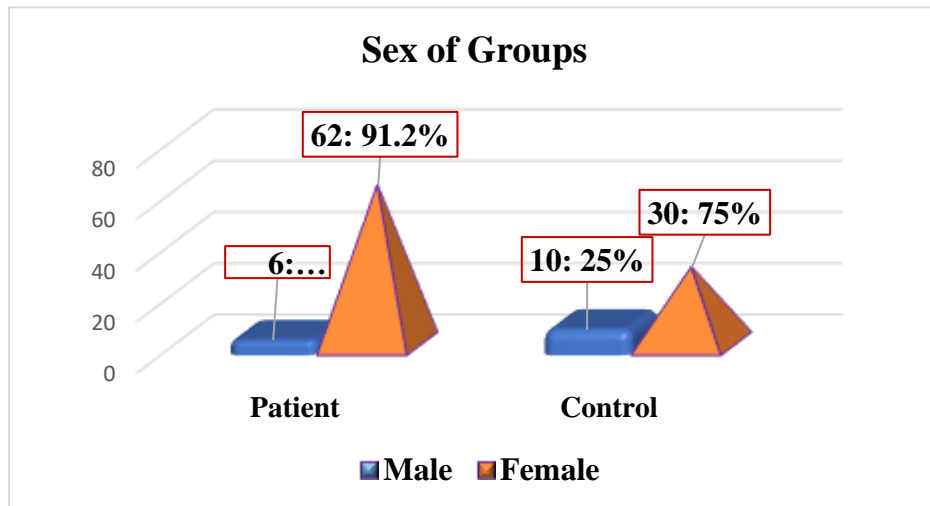
Rheumatoid arthritis is a relatively common disease in our country. The diagnosis of RA depends primarily on clinical manifestations of the disease, whereas serological tests provide an aid in terms of confirmation. This study showed that the predominant range age affected by RA was (41-50) years old this agreed with several studies (Al-Ubaidi et al., 2013), which demonstrated that RA develops after the age of 40 years due to a variety of factors, including thymic depression, Stress that lowers immunity, and exposure to other antigens, such as smoking (tobacco), which activates auto-reactive lymphocytes (Albaqami et al., 2019) in Saudi Arabia found mean of age was (49.7 ± 12.67) years, The current results are compatible or slightly different with several studies carried out in different countries including;

(Faiq et al., 2019) in Iraq who found the mean (50.8 ± 13.1) years, while the mean was a little high. in Columbia was (52.73 ± 12.8) years (Barragán- Martínez et al., 2012); While the mean was high in RA patients in China (55.3 ± 12.6) years (Liu et al., 2014). In France the mean was (56.05 ± 10.54) years (Soósová et al., 2017). Other studies indicated that the higher average mean age in Japan was 59.8 years, (Iwamoto et al., 2007), in California (58.0 ± 10.8) years as a study performed by (Katz et al., 2013), in Sweden (Turesson et al., 2016) found that age of RA patients was (58.0 ± 7.2) years. The current results disagree with (Al-Norri et al., 2020) in Iraq who found that highest percentages were in the age groups (31-40) years. These differences could be related to dissimilarities in time of diagnosis, wither diagnosed early or later in the advanced stage. While other studies in different countries in the world recorded the lowest percentages such as in India (Haroon et al., 2007) report that age of rheumatoid patients was (44.4 ± 10.5) years; (Hussain et al., 2016) in Saudi Arabia found (43.3 ± 12.0) years. The high percentages from the present study demonstrated by (Kushioka et al., 2018) in Japan (64.3 ± 9.4) years; (55.6 ± 13.0) years (Tong et

al., 2020) in China they report age of RA patients more than 60 years, these differences could be due to genetic and environmental factors.

B. Distribution of RA patient according gender:

(Figure-2) showed the result of distribution of groups according gender , in patient showed the female in (91.2%) in compared to male was (8.8%).while in healthy groups showed the female was(75%) and male was(25%) .Significant difference between female to male was highly significant difference(p.value<0.001).



$\text{CalX}^2= 9.072$ $\text{TalX}^2= 3.84$ $\text{DF}= 1$ $\text{p. value } 0.003$ S High-Significant

Figure 2. Distribution of RA patient according gender

The study results (according to gender) showed that the proportion of female to male with RA patient is (10.33:1) . The distribution of RA among patients in females was (91.2 %) compared with male was (8.8%) (Figure-2), This mean female at more risk than male, that compatible with (Noori ,2021) who mention the percentage of female was (82.4 %) while in male (17.6%) with a ratio (4.6:1),Also nearly with result of (Chiads ,2015) findings that (78.67%) of RA patients were females, and in agreement with recent studies in Iraq by El-Saffar (2008) and Al-Tayyar (2012) that note the prevalence of RA among females was 75% and 82%, respectively. Other studies (Kvien et al., 2006) found a female to male ratio in western countries at (4.5:1). An Egyptian study conducted by (Sakr et al., 2018) showed the ratio in female compared to males was (5.2:1) ; An Algerian study showed a ratio of female to male (5.9:1) (Slimani et al., 2014) . On other hand some studies demonstrated a less ratio of female: male (3:1) by (Soósová et al., 2017; Krasselt and Baerwald,2019; and Das et al, 2021). This differences in results may be due to some effects of hormones especially sex hormones that affect the immune system. A new study revealed that the pathogenesis of the disease and production of cytokines is depend on these differences between both sex and in consequences affect the activity of the disease and response to medication (Arif et al.,2023; Shepherd et al., 2019) . Increased rates of females with RA may be related to sex hormones and the female[w menstrual cycle (Gerosa et al.,2008; Islander et al.,2011; Alpízar-Rodríguez et al.,2017; Margiana et al.,2022) . The hormonal effects like estrogen, which promote the activity of T-helper cells while inhibiting the function of T-suppressor cells, hence may increase the onset of autoimmunity (Klareskog et al., 2008; Cutolo et al. (2002) found that the local immunological inflammatory response appears to be significantly influenced by the rise in estrogen level and the fall in androgen level in the RA synovium. Rheumatoid arthritis has an inverse association with androgen, which may explain the lower incidence of the disease in men (Mohammed and Qasim , 2021; Jasim et al.,2022; Intriago et al., 2019).

C. Estimation of TNF- α in patient and control

Sera tested for TNF- α by use of ELISA technique showed that mean for patient was (41.6 \pm 14.1), and in healthy group was(5.16 \pm 1.58) (Table-2) , The result showed significant difference between patient & healthy. The mean of TNF- α in male patient was (45.9 \pm 13.8) while in control was (4.88 \pm 1.48), in female patient was (41.2 \pm 13.7) while in control was (5.26 \pm 1.65) and p. value was high significant differences (p.value <0.001)(Table-1).

Table 1. Estimation of TNF- α in patient and control

Groups Parameters	TNF α pg/ml Mean \pm SD
Patients	41.6 \pm 14.1
Control	5.16 \pm 1.58
p. value	0.000**

p-value 0.000 High-Significant

The mean of TNF- α was (41.6 \pm 14.1) in patient , while in control was (5.16 \pm 1.58) This study agree with (Hadinedoushan et al.,2016) which reached to the serum levels of (TNF- α) were (5.21 \pm 1.69) while in the control group were (62.4 \pm 27.1) and the (p.value <0.005) show significant difference between patient and control that also agree with (Ying et al.,2019; Rohmah et al.,2022; Zadeh et al.,2022) who mention significant difference (p.value<0.005) between patient and control(AL-Khafaji,2008) mention the level of TNF- α were significantly higher in RA patients compared to control groups.

D. Estimation levels of TNF- α in patient according to gender

The levels of TNF- α according gender were in male patient(45.9 \pm 13.8) and control (4.88 \pm 1.48) and in female patient (41.2 \pm 13.7), while in control (5.26 \pm 1.65)(Table-2).

Table 2. Estimation of TNF- α in patient and control according gender

Test	TNF- α
Male-Patients	45.9 \pm 13.8 ^a
Male-Control	4.88 \pm 1.48 ^b
Female-Patient	41.2 \pm 13.7 ^a
Female-Control	5.26 \pm 1.65 ^b
p. value	0.000**Significant
LSD	10.7

- ✓ Similar letters indicate that there are non-significant differences, while the different letters indicate the presence of significant differences.

E-Signs &Symptoms of RA patient

In (figure-3) showed signs of RA in this study patient were swelling of joint, redness over the affected joints, Along with weariness, weakness, weight loss, and low-grade fever, morning stiffness persisting more than 30 minutes is another symptom.



Figure 3. signs and symptom of RA in the study patient

IV. Conclusion

The study conclude that RA may have a genetic role to its progression and may increased level of TNF- α is due to immune response during infection,also the levels of TNF showed the initiates of inflammation associated with disease The activity and severity of RA may be significantly influenced by TNF- α (Figure-3).

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