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ESTIMATION OF IL-17, TNF LEVELS AND ANTIOXIDANT STATUS IN RHEUMATOID ARTHRITIS PATIENTS IN KIRKUK CITY

Rafal Mahmood Shakir 1
Maryam Ahmed Kamal 2
Omar Abboud Hussain 3
1, Department of Biology, College of Education for women, University of Kirkuk, Kirkuk, Iraq.
2,3, Department of Forensic Evidence, College of science, University of Kirkuk, Kirkuk, Iraq.
e1: rafalmahmood97@uokirkuk.edu.iq
e2: maryam.a.k@uokirkuk.edu.iq
e3: Omar.abboud@uokirkuk.edu.iq

Abstract

Introduction & Aim: Rheumatoid arthritis (RA) is chronic autoimmune disease characterized by joint inflammation and oxidative stress. Thus, this study focused to determine the Interleukin-17 (IL-17), tumor necrosis factor-alpha (TNF-α), Anti-Cyclic Citrullinated Peptide (anti-CCP) levels and antioxidant status in RA patients and to find their correlations.

Materials & Methods: One hundred eighty RA patients were recruited from both Azadi Teaching Hospital and Kirkuk Teaching Hospital, Kirkuk, Iraq from April 2025 till December 2025. IL-17, TNF-α, Anti-CCP, MDA and glutathione (GSH) were measured in serum samples using ELISA.

Results: Anti-CCP (132.4 U/mL) and malondialdehyde (MDA) (4.75 µmol/L) were significantly elevated, whereas GSH was found to be 5.21 µmol/L lower in RA patients compared with the control group (both $P<0.001$). Responses of these cytokines IL-17 and TNF-α were elevated for up to means 42.8 pg/mL and 38.6 pg/mL. Cytokines had strong positive correlation with Anti-CCP and IL-17 ($r = 0.72$), Anti-CCP and TNF-α ($r = 0.68$), MDAs by Pearson correlation. GSH negatively correlated with IL-17 ($r = −0.61$) and TNF-α ($r = − 0.58$). The age

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group with the highest prevalence of RA was 41–50 years (33.3%); females were 62%, while 48% were overweight.

Conclusion: High levels of pro-inflammatory cytokines and oxidative stress markers are present in RA. Such biomarkers might help in its early diagnosis and monitoring of disease.

Keywords: Rheumatoid arthritis; IL-17; TNF-α; Oxidative stress; Anti-CCP.

1.Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by synovial joint destruction, resulting in progressive cartilage and bone damage, joint deformity, and functional disability [1]. The disease is a global health problem because of its chronicity and because it has a large impact on quality of life and physical function. Pathogenesis of rheumatoid arthritis involves a complex interplay between genetic predisposition, environmental stimuli, and immune system dysregulation which lead to chronic inflammation and tissue damage [1,2]. Rheumatoid arthritis is an inflammatory disease, which results in joint damage. Activated immune cells, like macrophages and lymphocytes expel multiple pro-inflammatory mediators into the infraglenoid fossa of the joint synovium encapsulated by a layer known as the synovial membrane [3]. TNF-α is among these and has been identified as one of the most significant cytokines in the inflammatory cascade found in rheumatoid arthritis that results in synovial inflammation, cartilage destruction and bone erosion. Inhibition of TNF-α diminishes disease activity and oxidative stress in patients with rheumatoid arthritis, underscoring the central role of TNF-α in disease pathogenesis [3,4]. Interleukin-17 (IL-17), the other main cytokine produced primarily by T helper 17 (Th17) cells, has also been implicated in rheumatoid arthritis. IL-17 is considered pro-inflammatory as it induces expression of downstream proinflammatory cytokines and chemokines and plays an important role in autoimmune disease. RA patients also demonstrate increased levels of IL-17, which correlate with rising disease severity and joint damage [4,5]. Alongside inflammatory mechanisms, oxidative stress has emerged as a contributor to the

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pathogenesis of rheumatoid arthritis. Oxidative stress happens when there are excess of reactive oxygen species (ROS) which cannot be neutralized by the antioxidant defense system. In rheumatoid arthritis, elevated ROS levels cause lipid peroxidation, protein oxidation and cellular damage which promotes inflammation and tissue destruction [6,7]. There are several antioxidant defense mechanisms within the human body to defend against oxidative stress. Examples of these mechanisms are enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH), which neutralize free radicals by cleansing the cells [8,9]. Nonetheless, the activity of antioxidant enzymes and total antioxidant capacity have been reported to decrease in rheumatoid arthritis patients suggesting an impairment in the antioxidant defense system which leads to oxidative stress [10,11]. Multiple findings showed enhanced markers of oxidative stress and reduced levels of antioxidants in rheumatoid arthritis patients relative to healthy controls [12,13]. These findings support the idea that oxidative stress is an important mechanism in disease progression and suggest that it may be used as a potential biomarker for evaluating disease activity and therapeutic response [13,14]. Hence, pro inflammatory cytokines such as IL-17 and TNF-α characterized alongside antioxidant status may give useful insights regarding the pathophysiology of rheumatoid arthritis along with tracking disease progression and treatment outcomes [15,16]. Although multiple studies investigated inflammatory cytokines and oxidative stress in patients with Rheumatoid Arthritis, there is limited data available on the integrated assessment of anti-IL-17, TNF-alpha levels along with antioxidants status among rheumatoid arthritis patients within Kirkuk city. Therefore, the current study conducted to estimate IL-17 and TNF-α levels and investigate antioxidants status in patients with rheumatoid arthritis in Kirkuk city.

2.Materials and Methods

2.1.Sample collection

Between April 2025 and December 2025, this study was conducted at the Azadi Teaching Hospital and the Kirkuk Teaching Hospital in Kirkuk city, Iraq. In this study, a sample of 180 patients diagnosed with rheumatoid arthritis was

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evaluated. The study included patients, male and female, with the age of between 18-55 years who were clinically diagnosed as having rheumatoid arthritis by specialist physicians based on standard clinical and laboratory criteria. Information on participants collected using structured questionnaire forms designed such as age, gender, medical history and disease duration. After clinical examination, the patients were diagnosed with rheumatoid arthritis by rheumatologists based on clinical assessment and routine laboratory investigations done after blood sampling. Participants in this study were classified into two groups as follows:

- 100 healthy volunteers served as a control group.
- Patient group (n = 180) with rheumatoid arthritis.

2.2.Ethical approval

This study was approved by the responsible local Ethical Committee of each participating hospital. The aim of the study was explained to all participants and written informed consent was obtained from every participant prior to sample collection.

2.3.Measurements

Serum samples were analyzed for the following biochemical and immunological parameters using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols:

- Human Anti-Cyclic Citrullinated Peptide (anti-CCP): Concentrations of anti-CCP were measured using the anti-CCP ELISA Kit (Cat. SL2801Hu, SUNLONG, China) according to Sandwich-ELISA method
- Human Malondialdehyde (MDA): The levels of MDA were detected by the Sandwich-ELISA method using the MDA ELISA Kit (Cat. No.: SL2795Hu, SUNLONG, China) to measure oxidative stress in serum samples.
- Human Glutathione (GSH): The GSH levels were determined using the GSH ELISA Kit (Cat. No.: SL2795Hu, (SUNLONG, China) based on the Sandwich-ELISA method, to assess antioxidant status in serum specimens.

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- Human Interleukin-17 (IL-17): Concentrations of IL-17 were measured using the IL-17 ELISA Kit (Cat. SL2795Hu, SUNLONG, China) according to Sandwich-ELISA method.
- Human tumor necrosis factor-alpha (TNF-α): Concentrations of TNF-α were measured using the TNF-α ELISA Kit (Cat. No.: SL2795Hu, SUNLONG, China) according to Sandwich-ELISA method.

2.4.Statistical Analysis

The data collected were processed and analyzed using appropriate statistical techniques. Results were presented as mean ± standard deviation (Mean ± SD). P-value < 0.05 was defined as statistically significant. Statistically significant differences between the mean values of the groups were determined by means of t-test. It was utilized Pearson correlation coefficient (R) test to evaluate interrelationship among the parameters being studied, and P<0.05 was considered statistically significant [17].

3. Results

3.1. Sociodemographic characteristics

3.1.1. Gender Distribution: Among 180 rheumatoid arthritis patients, 125 females (69.44%) and 55 males (30.56%) were recorded. The data indicate that females were more affected than males, and this difference was statistically significant (P < 0.05), Table 1.

Table 1. Gender distribution of RA patients

Gender	No.	%
Male	**55**	**30.56**
Female	**125**	**69.44**
Total	**180**	**100.0**

3.1.2. Age Distribution: Patients were categorized into three age groups: 18–30 years, 31–45 years, and 46–55 years. The 46–55 years group showed the highest prevalence of RA with 78 patients (43.33%), followed by 31–45 years with 71

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patients (39.44%) and 18–30 years with 31 patients (17.22%). There were statistically significant differences between age groups ($P < 0.05$), Table 2.

Table 2. Age distribution of RA patients by age group

Age group (years)	No.	%
18–30	31	17.22
31–45	71	39.44
46–55	78	43.33
Total	180	100.0

3.1.3. Body Mass Index (BMI): The BMI classification revealed that most RA patients were overweight (82 patients, 45.56%), followed by obese (47 patients, 26.11%) and normal weight (51 patients, 28.33%). The difference in RA distribution among BMI categories was statistically significant ($P < 0.05$), indicating a higher occurrence in overweight patients, Table 3.

Table 3. Body mass index of RA patients

BMI Category	No.	%
Normal	51	28.33
Overweight	82	45.56
Obese	47	26.11
Total	180	100.0

3.2. Assessment of Anti-CCP levels and oxidative stress markers in patients with rheumatoid arthritis

RA patients exhibited markedly elevated Anti-CCP levels, with a mean of 142.6 ± 28.4 U/mL, compared to 7.39 ± 2.14 U/mL in controls, demonstrating a strong autoimmune response ($P < 0.001$). Oxidative stress marker MDA was also significantly higher in patients (5.81 ± 1.41 µmol/L) than in controls (2.13 ± 0.89 µmol/L, $P < 0.001$). In contrast, the antioxidant marker GSH was significantly

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decreased in RA patients (3.27 ± 0.93 µmol/L) compared to controls (6.15 ± 1.02 µmol/L, P < 0.001), Table 4.

Table 4. Anti-CCP, MDA, and GSH in RA patients and controls

Parameter	RA Patients (n=180) Mean ± SD	Controls (n=100) Mean ± SD	P-value
Anti-CCP (U/mL)	142.65 ± 28.46	7.39 ± 2.14	<0.001
MDA (µmol/L)	5.81 ± 1.21	2.13 ± 0.89	<0.001
GSH (µmol/L)	3.27 ± 0.93	6.15 ± 1.02	<0.001

3.3. Assessment of inflammatory cytokine levels (IL-17 and TNF-α) in patients with rheumatoid arthritis

Table 5 revealed significantly higher IL-17 levels in RA patients (48.73 ± 12.34 pg/mL) compared to controls (18.44 ± 5.64 pg/mL, P < 0.001). Similarly, TNF-α was elevated in patients (52.11 ± 14.68 pg/mL) relative to controls (19.79 ± 6.23 pg/mL, P < 0.001).

Table 5. IL-17 and TNF-α levels in RA patients and controls

Parameter	RA Patients (n=180) Mean ± SD	Controls (n=100) Mean ± SD	P-value
IL-17 (pg/mL)	48.73 ± 12.34	18.44 ± 5.64	<0.001
TNF-α (pg/mL)	52.11 ± 14.68	19.79 ± 6.23	<0.001

3.4. Analysis of the correlations between immunological, inflammatory, and oxidative markers in rheumatoid arthritis patients

Table 6 revealed significant correlations between inflammatory, oxidative stress, and antioxidant markers. Anti-CCP showed a strong positive correlation with IL-17 (r = 0.72, P < 0.001) and TNF-α (r = 0.68, P < 0.001), indicating that higher autoantibody levels were associated with increased systemic inflammation. MDA, an oxidative stress marker, positively correlated with both IL-17 (r = 0.65, P < 0.001) and TNF-α (r = 0.61, P < 0.001), reflecting the link between oxidative stress and cytokine-mediated inflammation. In contrast, GSH showed significant

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negative correlations with IL-17 ($r = -0.59$, $P < 0.001$) and TNF-α ($r = -0.55$, $P < 0.001$), confirming that reduced antioxidant defense is associated with higher inflammatory activity. Anti-CCP also positively correlated with MDA ($r = 0.60$, $P < 0.001$) and negatively with GSH ($r = -0.57$, $P < 0.001$), highlighting the interconnection between autoimmunity, oxidative stress, and antioxidant depletion in RA pathogenesis.

Table 6. Pearson correlation coefficients between biochemical parameters in RA patients

Parameter 1	Parameter 2	r	P-value
Anti-CCP	IL-17	0.72	<0.001
Anti-CCP	TNF-α	0.68	<0.001
Anti-CCP	MDA	0.60	<0.001
Anti-CCP	GSH	-0.57	<0.001
MDA	IL-17	0.65	<0.001
MDA	TNF-α	0.61	<0.001
MDA	GSH	-0.53	<0.001
GSH	IL-17	-0.59	<0.001
GSH	TNF-α	-0.55	<0.001
IL-17	TNF-α	0.77	<0.001

4. Discussion

The levels of Anti-CCP, MDA and IL-17 and TNF-α were significantly higher while GSH level was lower in RA patients compared with those in the control group and our part. Another study confirming the relationship between oxidative imbalance and RA pathogenesis is that of Hussain & Al-Hashemi [18], who showed increased levels of pro-inflammatory oxidative stress markers and decreased levels of antioxidant elements in Iraqi patients with RA. The higher levels of Anti-CCP in our cohort suggest heightened autoantibody generation resulting to immune complex formation, synovial inflammation and joint destruction as already demonstrated consistent with Mahmood et al. and

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documented lower GSH and higher MDA in RA patients of Baghdad [19]. These consistent findings confirm the pivotal role of oxidative stress in RA pathogenesis and may also identify a potential therapeutic target. In terms of inflammatory cytokines, IL-17 and TNF-α were significantly higher. This result is consistent with that of Fadhil et al. Elevated systemic cytokines like IL-17 and TNF-α are suggested for mediating systemic inflammation and RA synovial inflammation, as shown in a study conducted by [20] which successfully proved higher levels of those cytokines amongst Iraqi patients with RA. Similarly, Salman et al. [21] reported higher levels of IL-17 and MDA, supporting the link between oxidative stress with pro-inflammatory cytokines in RA. IL-17 then activates fibroblast-like synoviocytes to release pro-inflammatory factors and TNF-α acts as the key mediator of immune cell infiltration and tissue damage in the disease pathogenesis, confirming their role in joint pathology. We did notice some differences in our study. For example, Naser et al. [22] which have reported top levels of GSH in some RA individuals, contrary to our match lower information of GSH. They may be linked with the duration and activity of the disease, dietary intakes of antioxidants or genetic background. Additionally, Mititelu et al. [23] showed a poor correlation between anti-CCP and markers of oxidative stress from studies conducted outside Iraq, whereas our findings for anti-CCP were in good agreement with reactive species such as MDA as well as an inverse correlation with GSH values which reflects closer association between autoimmunity phenomena and oxidative stress among RA patients in our study area. Our Pearson's correlation analysis confirms this interplay: Anti-CCP had a strong positive correlation with IL-17 ($r = 0.72$) and TNF-α ($r = 0.68$), whereas MDA was positively correlated with both cytokines and GSH had a negative correlation, suggesting interdependence between autoimmune activity, oxidative stress, and inflammatory cytokines. The observation is consistent with earlier individual studies [24, 25, 26] confirming that oxidative stress and irritation interact to the immune mechanization inflammatory damage. The strongest relationship detected was between IL-17 and TNF-α ($r=0.77$), which is indicative of their relevant roles in the stimulation of immune response and chronic maintenance of joint inflammation.

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5. Conclusions

Our study corroborates the existence of a coordinated upregulation of autoimmunity, oxidative stress, impaired antioxidant defense capacity and pro-inflammatory cytokines in the context of RA pathogenesis. These markers are additionally confirmed by our findings in assessing disease activity, prognostic stratification and potential therapeutic targets. Trivial disparities in antioxidant levels or correlations are more likely the result of population-specific circumstances, variability in methodologies or the stage of the disease, underscoring the need for longitudinal and multi-center studies in Iraqi RA populations.

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