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RESEARCH ARTICLE OPEN ACCESS

Biochemical Characteristics of Rheumatoid Arthritis among Iraqis: A Gender-Matched Comparative Study

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Abstract
Objective: This study aimed to assess the biochemical characteristics of rheumatoid arthritis among male and female Iraqis.
Methods: A total of 53 individuals aged 25–69 years were enrolled from Alrefia General Hospital in Thi-Qar. Among these, 33 patients diagnosed with rheumatoid arthritis (12 males and 21 females) and 20 age- and gender-matched healthy controls (14 males and 6 females) underwent evaluation. Serum uric acid and alkaline phosphatase levels were measured, and rheumatoid factor tests were conducted
Results: The results showed that out of the female participants, 21 were positive and 6 were negative cases. For the male participants, 26 were positive cases, and 14 were negative. There was a 2:1 ratio of females to males with rheumatoid arthritis.
Conclusion: Rheumatoid arthritis patients have greater levels of uric acid and alkaline phosphate in their blood, and the condition is more common in females than in males. It is important to note that patients' rheumatoid factors vary.

Keywords: Rheumatoid arthritis, Alkaline phosphatase, Uric acid, Rheumatoid factor

Plain English Summary

This research studied chemical compounds, reactions, and the process of developing rheumatoid arthritis among male and female Iraqis. Study participants were males and females between 25 and 69 years. The research enrolled 53 patients with rheumatoid and 20 control patients. A blood test to measure an enzyme in the bone, alkaline phosphatase, to diagnose bone disorder was carried out among the two groups. Also, the level of uric acid, a waste product found in the blood, was assessed in the two groups at Al-Rifai Hospital. Out of the female participants enrolled, 21 and 6 were positive and negative for the tests, respectively. Among the male participants, 26 and 14 were positive and negative for the tests, respectively. This study concluded that rheumatoid arthritis patients have higher levels of uric acid and alkaline phosphate in their blood, and the condition is more common in females than in males.

Background

Autoimmunity refers to the failure of the body's mechanisms that maintain self-tolerance and trigger a targeted immune response against its components. As a result, B and T cells produce antibodies targeting autoantigens, which are

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normal parts of an individual. These components are referred to as autoantigens or self-antigens. These components usually consist of proteins or proteins bonded to nucleic acids. Autoantibodies are the immune system-produced proteins that search for and attach to self-antigens. Similarly, T lymphocytes that respond to self-antigens are termed autoreactive T-cells (1).

Arthritis, derived from the Greek artho (joint) and itis (inflammation), is a form of joint disease in which one or more joints become inflamed. There are about 100 types of arthritis. Degenerative joint disease, the most common type of osteoarthritis, is brought on by age, joint infection, or joint trauma. Psoriatic arthritis, rheumatoid arthritis, and related autoimmune diseases are other types of arthritis. Joint infections are the cause of septic arthritis (1). Rheumatoid arthritis (RA) is a chronic disease of unknown cause that mostly affects synovial joints. It is a systemic autoimmune disease that is defined by extra-articular involvement and inflammatory arthritis. It is frequently symmetrical, begins in minor peripheral joints and progresses to proximal joints if therapy is not received. Early RA is defined by symptoms that go away in less than six months, while established RA is defined by symptoms that disappear in more than six months (2).

Over time, joint inflammation causes the joint to deteriorate with cartilage and bone degradation. The lack of a pathological laboratory test for rheumatoid arthritis complicates its diagnosis. A thorough and focused clinical strategy is necessary to make a diagnosis and avoid important joint injury (3).

Patients with rheumatoid arthritis need treatment via both pharmacological and non-pharmacological methods. The outer layer of the cell membrane contains a type of isozymes called alkaline phosphatases (ALPs). Their physiochemical properties are varied, and they boost the hydrolysis of organic phosphate esters found in the extracellular environment, such as zinc. Because they catalyze the same process, they are true isoenzymes. Alkaline phosphatase is a cytosolic enzyme that is found in the hepatocyte's canalicular membrane (4). This study aims to assess biochemical characteristics of Rheumatoid Arthritis among male and female Iraqis.

Material and Methods

Study design

A total of 53 participants were recruited from Alrefia General Hospital in Thi-Qar, aged 25 to 69 years. Among these, 33 patients were clinically diagnosed with rheumatoid arthritis (12 males and 21 females), and 20 healthy individuals served as controls (14 males and 6 females).

Estimation of Uric acid

Principle: Allantoin, carbon dioxide, and hydrogen peroxide are produced when uricase reacts with uric acid. Quinonimine is a red complex product that is produced when hydrogen peroxide and peroxidase react with the chromogen (amino-antipyrine and dichloro-hydroxybenzene sulfonate). A measurement of absorbance at 520 nm (490-530) indicates how much uric acid is present in the sample (according to the manufacturer, Biolabo, France).

Test procedure

Table 1: Let stand reagents and specimens at room temperature

	Automated analyzer	Manual Procedure
Reagent 1	240 µL	800 µL
Standard / Control or Specimen (1)	8 µL	25 µL
Reagent 2	60 µL	200 µL

Mix. Let stand for 300 sec at 37°C. Record absorbance at 505 nm versus reagent blank. Reaction is steady for 30 minutes

Calculation:

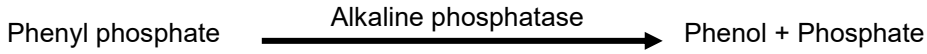
$$U.A = \frac{\text{Abs (assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Table (2): Normal values (According to manufacturer of kit)

Serum/Plasma	N.V Mg/dl
Child	[2.0-.5.5]
Men	[3.5-7.2]
Women	[2.6-6.0]

Estimation of Alkaline Phosphatase (Principle)

Colorimetric determination of the ALP activity, which reaction scheme of which is as follows:



The ALP activity in the specimen is directly proportional to the absorbance at 510 nm of the red-colored complex that is formed when 4-amino-antipyrine and free phenol, which is produced after substrate hydrolysis, react with alkaline potassium ferricyanide. The reagent's sodium arsenate eliminates further enzyme activity and stops the color dilution that comes with using older techniques (according to the manufacturer, Biolabo, France).

Test procedure

Table 3: Let stand reagents and specimens at room temperature

Prepare tubes as follows	Blank	Specimen blank	Standard	sample
Reagent R1	2 mL	2 mL	2 mL	2 mL
Incubate for five minutes at 37°C.				
Specimen				50 µL
(Standard)			50 µL	
Let stand for exactly 15 minutes at 37°C.				
Reagent R2	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Mix well.				
Reagent R3	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Demineralized water	50 µL			

Mix. Incubate 10 minutes at normal temperature away from light. Compare the absorbance of the assay, standard, specimen, and blank at 510 nm to the reagent blank. Coloration is consistent for 45 minutes apart from light

Calculation:

$$\text{ALP activity} = \frac{\text{Abs Assay. Abs Specimen blank}}{\text{Abs Standard}} \times 20$$

Table 4: Normal values (According to manufacturer of kit)

Serum/Plasma	N.V IU/L (37°C)
Birth	[36.107]
1 month	[71.213]
3 years	[71.142]
10 years	[107.213]
Adults	[32.92]

Statistical analysis

Data were analyzed using SPSS version 25. All continuous variables were first assessed for normality using the Shapiro-Wilk test. For variables that demonstrated a normal distribution—such as serum uric acid and alkaline phosphatase levels—the independent-sample t-test (two-tailed) was used to compare the patient and control groups. Variables that did not meet the normality assumption were analyzed using the Mann-Whitney U test. A p-value ≤ 0.05 was considered indicative of statistical significance.

Results & Discussion

A total of 53 individuals participated in the study. The RA group comprised 33 patients (12 males and 21 females), and the control group included 20 healthy subjects (14 males and 6 females). Figure 1 displays the demographic characteristics of the study population.

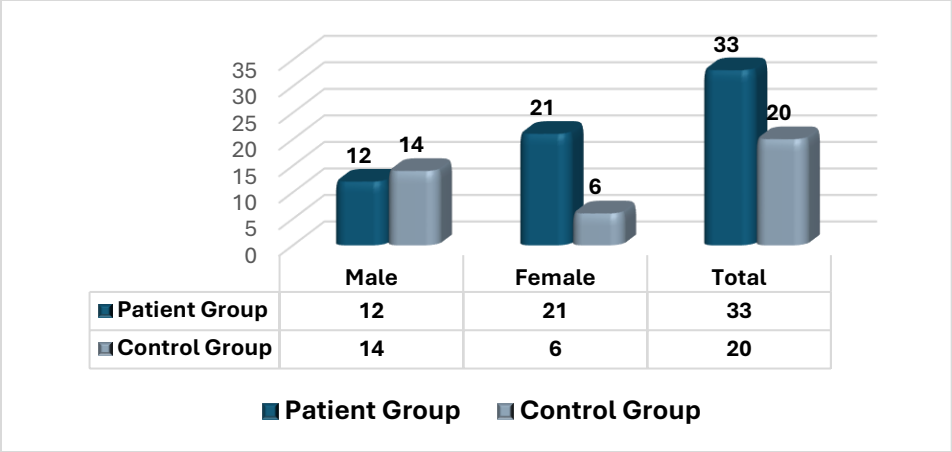


Figure 1: Demographic Characteristics of Study Participants

With an incidence ratio of 2:1 to 3:1, RA was more prevalent in women than in males. This was consistent with Venetsanopoulou *et al.* (5), which confirmed that RA affects women at a rate of 3:1 compared to men. The higher prevalence of the condition in females suggests that reproductive and hormonal factors may contribute to its susceptibility, development, and persistence. Studies on sex hormones, such as androgens and estrogens, are becoming more and more common, even though it is still unclear if they affect the course of the illness. According to Chang *et al*. (6), androgens have been shown to have a greater anti-inflammatory effect, whilst estrogens have a higher pro-inflammatory effect.

The biochemical analyses revealed that the mean serum uric acid level in RA patients was 9.42 ± 2.39 mg/dL compared with 5.26 ± 1.01 mg/dL in the control group ($p < 0.001$). Table 5 summarizes these findings.

Table 5: Comparison of Serum Uric Acid Levels between RA Patients and Controls

Variable		Patient Groups	Control Groups	P-Value
Uric acid (mg/dl)	Standard deviation	2.39	1.01	<0.001
	Minimum	6.60	3.10	
	Maximum	15.2	7.00	

In table (5), the p-value (<0.001) indicates that the uric acid levels in the patient group (case) (mean ± SD = 9.42 ± 2.39) were significantly greater than the control group's value (mean ± SD = 5.26 ± 1.01) and It is consistent with the study of Nada *et al.* (7) confirmed that more than 90% of RA patients had high serum uric acid levels.

Raised SUA levels in RA patients are not associated with HT, according to this study, suggesting a direct association that cannot be explained by other HT variables, including age, sex, obesity, or dyslipidemia. People with hypertension usually exhibit high levels of uric acid (SUA). SUA is elevated in 47.5% of persons with malignant hypertension or renal failure, 50% of those taking diuretics, and 25% of untreated hypertensives. It was found that reactive hyperemia and forearm blood flow measurements, two markers of microvascular function, are inversely correlated with SUA levels in hypertensive people. The result agrees with Panoulas *et al.* (8) in their study. Thus, demonstrating a connection between elevated SUA and dysfunction of endothelial cells in hypertensive patients could suggest non-dipping nocturnal blood pressure and an increased chance of cardiovascular disease. It is the first research to show that uric acid levels might play a role in the cause of HT in patients with RA. It is interesting to note that this link exists regardless of the medications these individuals frequently take or the underlying systemic inflammation.

Similarly, the analysis of alkaline phosphatase levels showed that RA patients had significantly higher levels (170 ± 17.3 IU/L) than controls (81.2 ± 10.6 IU/L), with a p-value <0.001. These results are detailed in Table 6.

Table (6): Comparison of Serum Alkaline Phosphatase Levels between RA Patients and Controls

Variable		Patient Groups	Control Groups	P-Value
Alkaline Phosphatase (U/L)	Mean	170	81.2	<0.001
	Standard deviation	17.3	10.6	
	Minimum	142	67.0	
	Maximum	200	101	

The data in Table 6 indicates an important increase in alkaline phosphatase in the patient group (mean ± SD = 170 ± 17.3) compared to the control group (mean ± SD = 81.2 ± 10.6). This study confirms the findings of other authors (9).

Patients with rheumatoid arthritis frequently exhibit elevated alkaline phosphatase because of degenerative changes in their wrist and other minor joints, which cause autoimmune erosive alterations in bone. Osteoblastic activity, a sign of increased bone turnover, has been linked to elevated ALP in RA. One characteristic of RA is increased bone turnover. The process of bone metabolism consists of the creation and resorption of bones. Both elements continuously follow one another throughout this process. Any difference between these processes could result in increased or reduced bone material density as the probability of issues as mentioned by Janković *et al.* (10). An inflammation-causing condition is characterized by chronic inflammation of the synovium, particularly in small joints. It often causes articular cartilage and juxta-articular bone to be destroyed. It agrees with the findings of Hitchon & El-Gabalawy (11). Greater alkaline phosphatases in patients are associated with greater resorption of bone formation, which is also facilitated by cytokines. Additionally, it is recognized that increasing disease activity causes more active bone resorption, which raises serum ALP and causes bone formation, as mentioned by Orsini *et al.* (12). ALP leaking from damaged or dead cells may be the cause of increased activity. The body begins to rebuild the damaged bone in the joints as a compensatory strategy for the alterations in bone, which causes osteoblasts to proliferate and raise alkaline phosphatase activity. Because the autoimmune destruction of rheumatoid arthritis makes the already-damaged bone brittle, increasing the patient's risk of fractures, it can serve as an excellent environment for osteoporosis to develop (13).

Rheumatoid factor

In individuals with musculoskeletal pain but no joint swelling, RF testing is not a screening test for RA. Autoantibodies are linked with both genetic and environmental risk factors for RA, and this is what Scherer *et al.* (14) pointed out. RA sensitivity is also decreased since RF is not present in all RA patients, and its specificity is limited because it is present in many disorders other than RA, which was confirmed by Soroush *et al.* (15) in their study. The test's predictive value is roughly 16% in patients with widespread musculoskeletal pain who do not have joint swelling. The predictive value of the RF factor rises to 80% in individuals with symmetric polyarticular joint edema, which is a high pretest risk of RA, which was confirmed by Mies Richie & Francis (9). RF is a significant indicator of a bad prognosis for RA patients. It predicts more joint degradation, extra-articular symptoms, and increased disability with high titers. Rheumatoid nodules are caused by rheumatoid factors that accumulate in tissue. Approximately 70% of RA patients have a positive RF test at the start of their disease, and 85% test positive within the first two years. It is not possible to track disease activity using serum levels of RF since they do not fluctuate quickly (16).

The present study demonstrated that RA patients exhibit significantly elevated serum uric acid and alkaline phosphatase levels when compared with healthy controls. The increased uric acid levels may be indicative of enhanced oxidative stress and an amplified inflammatory response associated with RA pathogenesis (17). Likewise, the higher ALP levels likely reflect augmented bone remodeling and osteoblastic activity that occur as compensatory responses to chronic joint inflammation (18). These biochemical alterations offer important insights into the metabolic disturbances present in RA and may contribute to our understanding of its progression.

It is important to note, however, that although the differences in these markers are statistically significant, the current study was not designed to establish diagnostic or prognostic cut-off values. The limited sample size precludes us from setting reliable thresholds for clinical use. Therefore, while the findings underscore key alterations in the biochemical milieu of RA patients, they should be interpreted with caution and regarded as preliminary evidence of metabolic dysregulation rather than as direct tools for diagnosis or monitoring.

Additionally, although elevated serum uric acid has been associated with hypertension in other

contexts (8), our data does not support a direct causal link between hyperuricemia and blood pressure alterations in the RA population studied. Rather, the hyperuricemia observed here may primarily represent a consequence of systemic inflammation rather than a standalone predictor of cardiovascular risk.

This study has several limitations that warrant consideration. First, the relatively small sample size (33 rheumatoid arthritis patients and 20 healthy controls) may limit the statistical power required to detect subtle differences in biochemical markers between groups. Without a formal sample size calculation performed before the study, there is an increased risk of type II errors, potentially overlooking modest yet clinically relevant associations. Consequently, the generalizability of our findings to the broader Iraqi population or other settings may be constrained. Future research should implement rigorous power analyses to determine adequate sample sizes, and multi-center studies with larger cohorts should be considered to validate these preliminary findings. Despite these limitations, our study provides valuable initial insights into the biochemical characteristics of rheumatoid arthritis among Iraqis.

Conclusion

This study confirms that RA patients have elevated serum uric acid (SUA) and alkaline phosphatase (ALP) levels compared to healthy controls. These findings underscore the biochemical alterations associated with RA; however, due to the limited sample size, further studies are warranted to explore their clinical utility and to establish diagnostic cut-off points.

List of Abbreviations

RA: Rheumatoid Arthritis
ALP: Alkaline Phosphatase
SUA: Serum Uric Acid
RF: Rheumatoid Factor
IU/L: International Units per Liter
mg/dL: Milligrams per Deciliter
SD: Standard Deviation
SPSS: Statistical Package for Social Sciences
T-cells: T Lymphocytes
B-cells: Lymphocytes
HT: Hypertension
DNA: Deoxyribonucleic Acid
Nm: Nanometer

Declarations

Ethical approval and consent to participate

This study was conducted following the principles outlined in the Declaration of Helsinki. Ethical approval for the research was obtained from the Ethical Committee of Alrefia General Hospital, Thi-Qar (approval number: [45632]). All participants provided written informed consent before their enrollment in the study. The confidentiality and anonymity of the subjects were strictly maintained throughout the research process.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Nil.

Author contributions

AAJ: Conceptualization, Methodology, Supervision, Writing – Original Draft. AMH: Data Curation, Laboratory Analysis, Investigation, Writing – Review and Editing. AMA: Statistical Analysis, Visualization, Validation, and Writing – Review and Editing.

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