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Association Between Serum Hepcidin Levels and Anemia in Patients with Rheumatoid Arthritis: A Case-Control Study

Doaa Faleh Mahdi[1], and Ameer Jabbar Shwayel[2]

[1] *Southern Technical University, Shatrah Technical College, Iraq.*

[2] *Thi-Qar Health Directorate, Shatrah General Hospital, Iraq.*

Email: DuaaFM12020mmgh0@stc.stu.edu.iq, ameeralzaidy9@gmail.com

ABSTRACT

Background: *Anemia is a prevalent comorbidity in rheumatoid arthritis (RA), primarily classified as anemia of chronic disease (ACD). Hepcidin, an iron-regulatory hormone induced by inflammation, is a key mediator in ACD pathogenesis, but its relationship with RA-specific autoimmunity remains to be fully elucidated.* ***Objective****: This study aimed to investigate the role of circulating hepcidin in RA-related anemia and its correlation with disease activity markers, including anti-cyclic citrullinated peptide (anti-CCP) antibodies.* ***Materials and Methods****: A case-control study was conducted from April to June 2025, comprising 50 newly-diagnosed RA patients and 50 healthy controls. Venous blood samples were collected to measure hematological parameters (hemoglobin, WBC), iron profiles (iron, ferritin), inflammatory biomarkers (CRP), RA serology (rheumatoid factor, anti-CCP), and serum hepcidin levels using ELISA and automated immunoassays.* ***Results****: RA patients exhibited significantly lower hemoglobin and serum iron levels, and significantly higher WBC, CRP, RF, anti-CCP, and hepcidin levels compared to controls ($p < 0.001$). Serum ferritin did not differ significantly. Patients with high anti-CCP levels (≥100 AU/mL) had significantly elevated hepcidin, CRP, RF, and WBC, and lower serum iron, indicating a strong association between anti-CCP titer, inflammatory activity, and iron dysregulation.* ***Conclusion****: Elevated hepcidin is significantly associated with anemia in RA patients. Levels of anti-CCP strongly correlate with increased inflammatory activity and hepcidin expression, suggesting that anti-CCP may be a useful marker for identifying RA patients with more active disease and a greater risk of developing hepcidin-mediated anemia.*

Keywords: Arthritis, Rheumatoid; Anemia; Hepcidins; Anti-Citrullinated Protein Antibodies; Biomarkers; Inflammation.

Article Information

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INTRODUCTION

The inflammatory condition known as RA is typified by symmetrical, long-term inflammation of both big and small joints. Neutral proteases and matrix metalloproteinases are released as part of the inflammatory process within the synovium, which causes the destruction of cartilage and periarticular bone (1). impacting approximately 1% of adults. The average point and period prevalence of RA were 51 in 10,000 and 56 in 10,000, respectively; however, the literature shows an extensive range of point and period prevalence depending on the population and data collection method (2).

The disease's feature is the proliferation of synoviocytes, particularly synovial fibroblasts, which leads to the degeneration of bones and joints. According to recent research, cytokines and other inflammatory mediators play a

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crucial part in the development of both the articular syndrome and a variety of other systemic symptoms of the illness. Specifically, one of the most prevalent signs of rheumatoid inflammation is anemia (3). One of the most prevalent comorbidities in RA patients is anemia. Actually, anemia of the kind that is typified by low blood iron levels combined with sufficient iron storage is commonly linked to RA and has been used as a model for chronic anemia (1).

The prevalence of RA is increasing annually, as is the frequency of RA that is accompanied by anemia. Anemia is a prevalent symptom of RA, and its occurrence in RA patients can reach up to 75.28 percent (4). The pathophysiology of chronic illness (anemia of inflammation) is complex and linked to immune system stimulation. Erythropoiesis is hampered by inflammation-induced immune-competent cell activation and increased proinflammatory cytokine release (partly because proinflammatory cytokines produce resistance to erythropoietin) (5). Iron metabolism is subsequently disturbed (in part because proinflammatory cytokines cause iron to be taken up by activated macrophages, rendering it inaccessible to iron-metabolizing cells) (6).

Hepcidin it is a type-II acute phase protein, and inflammatory cytokines, particularly interleukin (IL), stimulate its synthesis. Anemia, hypoxia, and inflammation all affect hepcidin expression, which is higher in inflammatory environments. By binding to the iron transporter ferroportin and causing its internalization and breakdown, hepcidin functions as both a negative iron-regulating hormone and an inflammatory mediator. Furthermore, because of its intimate connection to iron metabolism and inflammatory cytokines, hepcidin is regarded as a key mediator of ACD (7).

METHODOLOGY

Study Design and Population

A case-control study was conducted from April to June 2025 at Shatrah General Hospital, Thi-Qar, Iraq. A total of 100 participants were enrolled and divided into two groups: Patient Group (n=50): Individuals newly diagnosed with Rheumatoid Arthritis (RA) according to the established clinical criteria. Control Group (n=50): Age-matched healthy volunteers with no history of autoimmune, inflammatory, or hematological diseases. The study protocol was approved by the local institutional review board, and written informed consent was obtained from all participants prior to enrollment.

Inclusion and Exclusion Criteria

Inclusion criteria for the patient group consisted of adults (>18 years) with a new diagnosis of RA. Exclusion criteria for both groups included the presence of other autoimmune diseases (e.g., lupus), osteoporosis, hypocalcemia, malnutrition, liver or renal disorders, recent blood transfusions, pregnancy, or use of iron supplements or immunosuppressive drugs prior to sampling.

Sample Collection and Processing

A 5 mL venous blood sample was drawn from each participant via venipuncture of the median cubital vein. The sample was allocated as follows: 2 mL was collected in an EDTA tube for Complete Blood Count (CBC) analysis. 3 mL was collected in a serum gel tube. This tube was allowed to clot and then centrifuged at 3000 RPM for 15 minutes to separate the serum. The aliquoted serum was stored at -80°C until subsequent biochemical analysis.

Laboratory Assays

Laboratory assays were performed as follows: hemoglobin (Hb) and white blood cell (WBC) count were analyzed with an automated hematology analyzer (Sysmex XP-300);

C-reactive protein (CRP) titer, rheumatoid factor (RF) titer, and serum iron were quantified using a fully automated chemistry analyzer (Mindray BS-230); anti-cyclic citrullinated peptide (anti-CCP) antibodies and serum ferritin were measured via fluorescent immunoassay on an AFIAS-10 analyzer (Boditech Med Inc., Korea); and serum hepcidin levels were determined using a commercial ELISA kit (BT-LAB, Cat. No. E-EL-H6202) according to the manufacturer instructions.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software (version 23; IBM Corp.). Continuous data were presented as mean ± standard deviation (SD). The Student's t-test was used to compare means between the two groups for normally distributed data. The Chi-square (χ^2) test was used to compare categorical variables (e.g., sex distribution). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1 presents the baseline demographic profile of the study cohort, comprising 50 RA patients and 50 healthy control subjects. Statistical analysis using the t-test for age and the Chi-square test for sex distribution revealed no significant difference in the mean age between the patient and control groups (35.5 ± 8.1 vs. 34.4 ± 8.9 years, p=0.50), indicating successful age-matching and minimizing age as a confounding variable. However, a highly significant difference (p=0.001) was observed in sex distribution, with a female predominance in the RA group (80%) compared to the control group (50%).

Table 1: Demographic Characteristics of the Study Participants.

Variables		Patients NO.=50	Control NO.=50	P.value
Age (years)	**Children**	35.5± 8.1	34.4±8.9	0.50
Sex	**Males**	10	25	0.001
	Females	40	25	

t-test and Chi-square test were used

Table 2 compares key hematological and inflammatory parameters between RA patients and healthy controls. The results demonstrate significant dysregulation in the patient group. RA patients exhibited severe anemia, indicated by significantly lower hemoglobin levels (7.4 ± 1.2 g/dL vs. 13.4 ± 1.3 g/dL, p<0.001) and lower serum iron (6.22 ± 2.67 µmol/L vs. 10.15 ± 4.38 µmol/L, p<0.001), alongside elevated markers of systemic inflammation, including WBC count (10.75 ± 2.87 vs. 7.03 ± 2.11 x10⁹/L, p<0.001), CRP (49.78 ± 12.46 vs. 5.73 ± 2.79 mg/L, p<0.001), and RF titer (54.8 ± 10.8 vs. 5.0 ± 2.87 U/mL, p<0.001). The specific RA autoantibody, anti-CCP, was markedly elevated in patients (134.6 ± 28.57 vs. 3.12 ± 0.32 AU/mL, p<0.001). Crucially, hepcidin levels were significantly higher in RA patients (15.12 ± 5.66 vs. 10.82 ± 3.88 ng/mL, p<0.001), supporting its role in the anemia of chronic disease. No significant difference was found in serum ferritin levels (p=0.13).

Table 2: Comparative Analysis of Hematological and Inflammatory Biomarkers in RA Patients and Healthy Controls.

Variables	Study groups	Mean∓SD	P.value
Hemoglobin (g/dl)	**Patients**	7.4±1.2	<0.001
	Control	13.4±1.3	
WBC count (×10^9/ L)	**Patients**	10.75±2.87	<0.001
	Control	7.03±2.11	
Serum Iron (µmol/L)	**Patients**	6.22±2.67	<0.001
	Control	10.15±4.38	
Serum Ferritin (ng/mL)	**Patients**	31.78±6.98	0.13
	Control	33.31±5.44	
CRP titer (mg/L)	**Patients**	49.78±12.46	<0.001
	Control	5.73±2.79	
RF titer (U/mL)	**Patients**	54.8±10.8	<0.001
	Control	5.0±2.87	
Anti-CCP (Au/mL)	**Patients**	134.6±28.57	<0.001
	Control	3.12±0.32	
Hepcidin (ng/ml)	**Patients**	15.12±5.66	<0.001
	Control	10.82±3.88	

Table 3 stratifies RA patients based on their anti-CCP antibody levels (Group A: <100 AU/mL, Group B: ≥100 AU/mL) to analyze its correlation with disease activity. Patients with high anti-CCP titers (Group B) displayed a more pronounced inflammatory state, evidenced by significantly higher WBC counts (p=0.01), CRP (p<0.001), and RF titers (p<0.001). This group also showed more severe disruption of iron homeostasis, with significantly lower serum iron levels (6.03 ± 1.11 vs. 7.65 ± 1.73 µmol/L, p=0.001) and significantly elevated hepcidin levels (17.85 ± 2.37 vs. 13.76 ± 4.55 ng/mL, p<0.001). Hemoglobin and serum ferritin levels did not differ significantly between the two groups. This analysis confirms that high anti-CCP levels are a strong indicator of greater inflammatory activity and hepcidin-mediated iron restriction, key drivers of anemia in RA
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Table 3: Association of Anti-CCP Antibody Levels with Disease Severity and Iron Metabolism Markers in RA Patients.

Variables	Group A <100	Group B ≥100	P.value
Hemoglobin (g/dl)	10.44±1.15	10.91±1.17	0.18
WBC count (×10^9/ L)	9.87±2.09	11.01±1.02	0.01
Serum Iron (µmol/L)	7.65±1.73	6.03±1.11	0.001
Serum Ferritin (ng/mL)	31.02±6.22	31.99±7.26	0.65
CRP titer (mg/L)	33±65±9.54	65±15.86	<0.001
RF titer (U/mL)	40.54±8.48	68.99±12.97	<0.001
Hepcidin (ng/ml)	13.76±4.55	17.85±2.37	<0.001

DISCUSSION

Rheumatoid arthritis is associated with multifactorial anemia. Anemia was defined as HB 11 g/dl in female patients and 12 g/dl in male patients with RA (8) . Anemia is the most prevalent hematological issue among RA patients, affecting almost 50% of them. However, little research has been done on the relationship between anemia and disability in RA patients (3). Significant decrease of Hb in patient compaired with control, our study agree with previous study (4,9). ACD, sometimes referred to as anemia of inflammation, is the main cause of the drop in hemoglobin in RA patients. This type of anemia, which is caused by several interconnected physiological processes, is prevalent in long-term inflammatory diseases like RA. First, there was a rise in Hepc production due to iron restriction. Reduced iron absorption from the gut and iron entrapment in macrophages are the results of inflammation, particularly interleukin-6 and IL-6, which induce the liver to create Hepc. Hepc binds to ferroportin, an iron transporter on intestinal cells and macrophages, to limit iron export. Despite having normal or high iron reserves, there is less iron accessible for erythropoiesis (functional iron deficit) (7,10,11).

The observation that there was no statistically significant difference in the mean age of RA patients and controls ($p = 0.50$) indicates that the two groups were age-matched, which lessens the possibility that age served as a confounding factor for the observed variations in laboratory parameters. This finding is consistent with earlier research (5). However, there were more females in the RA group, and the sex distribution was significantly different ($p = 0.001$). This result is consistent with long-standing epidemiological trends that indicate RA is more common in women, frequently with female-to-male ratios ranging from 2:1 to 4:1 (12). One possible contributing component is hormonal impacts, specifically the function of estrogen in regulating immunological responses. Sex hormones may therefore affect both baseline and disease-associated levels, therefore even while the difference in sex distribution represents the natural disease trend, it should still be taken into account when evaluating inflammatory and immune-related indicators (13).

According to the research, RA patients and healthy controls differ significantly in a number of hematological and biochemical markers. Hemoglobin levels were considerably lower in RA patients (7.4 ± 1.2 g/dL vs. 13.4 ± 1.3 g/dL, $p < 0.001$. This is in line wit ACD (14), where iron availability for red blood cell synthesis is limited by pro-inflammatory cytokines (IL-6, TNF-α) that affect erythropoiesis and change iron metabolism through hepcidin overexpression (15). The patients' low serum iron levels further support this, which is consistent with multiple research (16). A persistent inflammatory state was indicated by the patients' higher WBC counts (10.75 ± 2.87 vs. 7.03 ± 2.11, $p < 0.001$). This outcome is consistent with earlier research (17). The systemic immune activation brought on by RA's autoimmunity may be the cause of this leukocytosis. In line with earlier research, serum iron levels were considerably lower in RA patients ($p < 0.001$) but not statistically different in FER patients ($p = 0.13$) (5). Ferritin is an acute-phase reactant and an iron-storage protein. Ferritin levels frequently rise in inflammation even though low circulating iron is a defining feature of ACI. FER levels in the data seem maintained rather than raised, which could indicate early-stage iron dysregulation or modest disease activity (18).

Systemic inflammation was confirmed by the significantly higher levels of the inflammatory biomarker CRP in the patients (49.78 ± 12.46 mg/L vs. 5.73 ± 2.79, $p < 0.001$). There was also a significant increase in

RF levels (54.8 ± 10.8 U/mL vs. 5.0 ± 2.87, p < 0.001). While elevated RF confirms the diagnosis and is linked to more aggressive joint disease, high CRP supports active disease (19). Increases in Anti-CCP, RF, and CRP were in agreement with earlier research, and anti-CCP antibodies were significantly greater in RA patients (134.6 ± 28.57 vs. 3.12 ± 0.32, p < 0.001), which is consistent with their known high specificity for RA and predictive usefulness for erosive illness (16,17). Hepcidin levels were significantly higher in RA patients (15.12 ± 5.66 vs. 10.82 ± 3.88, p < 0.001) which agree with previous study (20), Anemia is probably caused by IL-6-driven production during chronic inflammation (17).

Group B (high Anti-CCP) had considerably higher CRP and RF titers, indicating that larger Anti-CCP titers are associated with more active illness and systemic inflammation 19. The association between autoantibody titers and inflammatory activity was further supported by the fact that Group B had higher WBC counts. Compared to 7.65 ± 1.73, Group B had considerably lower serum iron levels (6.03 ± 1.11 µmol/L, p = 0.001). Group B had a considerably higher level of hepcidin (17.85 ± 2.37 ng/mL vs. 13.76 ± 4.55, p < 0.001.(This implies that increased IL-6-mediated hepcidin elevation results from more acute or antibody-driven inflammation, exacerbating functional iron shortage (19) this indicate that Anti-ccp used to evaluate activity of disease (21).

CONCLUSION

In conclusion, this study demonstrates that serum hepcidin levels are significantly elevated in RA patients with anemia compared to healthy controls. The increased hepcidin, likely driven by interleukin-6-mediated inflammation, contributes to functional iron deficiency and anemia of chronic disease (ACD) in RA. These findings underscore the role of hepcidin as a key mediator linking inflammation and anemia in RA and suggest that anti-CCP may serve as a useful biomarker for assessing disease severity and inflammatory burden. Further longitudinal studies are warranted to explore the potential of hepcidin as a therapeutic target in RA-related anemia..

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