A STUDY OF SERUM HEPCIDIN LEVEL IN RELATION TO CLINICAL ACTIVITY INDICES AND COMPLETE BLOOD PICTURE PARAMETERS IN RHEUMATOID ARTHRITIS PATIENTS

Sherihan M. Salama[1], Samia M. Rashad[1], Aya M. Elgendy[2], Heba S. Agamy[3] and Mona M. El-Bably[1]

[1]Departments of Physical Medicine, Rheumatology & Rehabilitation, [2]Internal medicine and Rheumatology and [3] Clinical pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Corresponding author:
Sherihan Mahdy Salama
Mobile: +2 01090604024

E-mail:
shrehan.mahdy@med.asu.edu.eg

Received: 06/02/2024
Accepted: 15/03/2024

Online ISSN: 2735-3540

ABSTRACT:

Background: *Rheumatoid arthritis (RA) is a chronic auto-immune-mediated inflammatory arthritis that affects around one percent of the human population. One of the common comorbidities encountered in rheumatoid patients is anemia. Hepcidin being one of the acute-phase reactant proteins, it acts as a homeostatic regulator of iron metabolism and as an inflammatory mediator as well.*

Aim of The Work: *This study aims to evaluate the value of measuring serum hepcidin levels in patients with rheumatoid arthritis and its relationship with clinical disease activity and blood parameters.*

Methods: *fifty RA patients underwent full medical assessment with the evaluation of DAS-28 score, laboratory tests including CBC, ESR, rheumatoid factor and anti-CCP, serum ferritin and serum iron, and serum hepcidin level.*

Results: *Our results showed that there was a statistically significant correlation between inflammatory markers and serum hepcidin level, as well as a positive correlation between the degree of disease activity measured by DAS 28 score with serum hepcidin level.*

Conclusion*: We concluded from our study that serum hepcidin can be used to assess the disease activity in rheumatoid arthritis patients and rheumatoid-related anemias as well.*

Keywords: *Rheumatoid arthritis, hemoglobin, anemia, hepcidin, anemia of chronic disease.*

INTRODUCTION:

Rheumatoid arthritis is the prototype of immune-mediated inflammatory diseases featuring functional affection of various organs including joints, kidneys, and many others where the affected joints suffer from proliferative synovitis leading to cartilage damage that occurs irreversibly [(1)]. The disease usually alternates between an active stage and a remission stage along the course of the disease. The determinants of disease activity and inflammatory milieu are the Disease Activity Score, routine laboratory tests involving complete blood picture, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) [(2)].

Different types of anemia could occur in immune diseases. Talking about rheumatoid arthritis, anemia is a common comorbidity that most commonly happens in the form of anemia of chronic diseases (ACD) or iron deficiency anemia (IDA). The development of ACD in RA patients is a sequel of immune system activation and cytokine production that is reflected in many aspects of hematological cells synthesis as homeostasis of iron, proliferation of erythroid progenitor cells, red cells life span, and the secretion of erythropoietin hormone [(3)]. Proinflammatory cytokines that were encountered to play a role in the development and progression of anemia include TNF alpha, interferon gamma, interleukin 1, and interleukin 6 [(4)].

On the other hand, IDA occurs in RA patients mostly due to gastrointestinal bleeding that could be attributed to prolonged treatment use and due to malabsorption [5].

Hepcidin is one of the acute-phase reactant proteins that is fully developed inside the liver cells. This peptide functions primarily as an inflammatory mediator in addition to being a homeostatic regulator of iron metabolism (by controlling iron release and mobilization in hepatocytes, macrophages, etc.) [6].

AIM OF THE WORK:

Our study aims to evaluate the value of measuring serum hepcidin levels in rheumatoid arthritis patients and its relationship with the clinical activity of the disease and blood parameters of the patients.

PATIENTS AND METHODS:

Study design and setting:

this study is a cross-sectional one that was conducted at Ain Shams University hospitals over 3 months.

Participants:

This study included a convenience sample of 50 rheumatoid patients who were recruited over three months from the outpatient clinics and inpatient departments of the Ain Shams University hospitals. Before enrollment, all participants were instructed about study objectives, as well as giving formal written permission. The Research Ethics Committee granted its ethical approval (FMASU R 160/2023). In accordance with the updated Helsinki Declaration of Biomedical Ethics, confidentiality will be protected when handling the data of the patients.

Patients with RA who met the 2010 American College of Rheumatology ACR/European League Against Rheumatism categorization criteria for RA, were eligible for inclusion in this study [7]**.** Individuals with other rheumatological disorders were disqualified from this research, as well as those who suffered from hematologic diseases, malignancy, chronic renal or hepatic disease, and other autoimmune diseases, patients who received pulse steroids of month duration before sampling, acute illnesses or infection, or a history of blood transfusions within the three months before to sample. Pregnant patients were also excluded as growth factors during pregnancy may markedly affect activity indices and blood picture.

All patients underwent:

A. Full medical history taking with special consideration for the disease onset and duration and treatment received in the last 6 months.
B. Clinical assessment including general and musculoskeletal examination.
C. Clinical evaluation of RA disease activity by using the modified disease activity score (DAS-28) which included the number of tender and swollen joints affected together with the patient's ESR and patient global assessment on the visual analogue scale (VAS). The score was calculated and was graded as remission, low, moderate and high disease activity according to the score (<2.6, >2.6-3.2, >3.2-5.1 and >5.1 respectively) [8].
D. Blood sample: Five-milliliter blood samples were collected by venipuncture under complete aseptic conditions. Samples were left to clot and then centrifuged at 1000×g for 15 min, Sera were used to measure serum levels of total Rheumatoid factor, anti-CCP, and other blood chemistry tests.
E. Another Five-milliliter blood sample was drawn directly into a buffered sodium citrate tube for ESR, and an EDTA tube for CBC, mixed right away, and analyzed in less than two hours. The following

parameters were detected; **(1)** Complete blood count and different blood indices were estimated on the day of sampling as fresh blood with no storage using an automated blood cell counter (Sysmex, Japan) and this included RBCs count, Platelets count, WBCs count, Hb, HCT, MCV, MCH, RDW, **(2)** Screening tests for diagnosis and activity detection of RA disease based on ESR levels using the Westergren methods. **(3)** Rheumatoid factor (RF) using Immunoturbidimetric assay on (Cobas 6000 c501 (Roche Diagnostics, Switzerland) and Anti-CCP test on Cobas e411 (Roche Diagnostics, Switzerland) **(4)** Fasting blood sugar together with liver and kidney function tests using an automated blood chemistry analyzer **(5)** Measurement of serum Ferritin and serum iron; Serum ferritin concentration was assessed by electrochemiluminescence immunoassay (ECLIA) on Cobas e411 analyzer (Roche Diagnostics, Switzerland) using a commercial kit for ferritin from Roche Diagnostic. Quantitative serum iron determination was measured on Beckman Coultar AU 680 Inc, USA) using a colorimetric method (intra-assay CV of 4%, inter-assay CV 6%), **(6)** The serum hepcidin level was measured using a commercially available ELISA kit (Bioassay Technology Laboratory, BT LAB-419 Shanghai, Korain, Biotech Co., Ltd, China), according to the manufacturer's instructions. Serum hepcidin concentrations were evaluated using an ELISA commercial kit for hepcidin (Uscn Life Science Inc. Wuhan, China). The sensitivity of the method was 0.05 ng/ml, and the detection range was between 0.188 and 12 ng/ml.

Statistical analysis:

The 28th release of IBM Corp.'s SPSS program, which was released in 2021, was used to evaluate the acquired data. Version 28.0 of IBM SPSS Statistics for Windows. IBM Inc., Armonk, New York Quantitative variables were defined using means and standard deviations, whilst categorical variables were reported using absolute frequencies and compared using the chi-square test. The trend test chi-square was used to ordinal binary data. To confirm the assumptions for parametric testing, Levene (homogeneity of variances) and Kolmogorov-Smirnov (distribution-type) tests were used. The independent sample t-test (for normally distributed data) and Mann-Whitney test (for not normally distributed data) were used to compare quantitative data between the two groups. The strength of the link between two continuous, non-normally distributed variables was evaluated using the Spearman rank correlation coefficient. To determine the likelihood that specific risk variables will result in specific health issues, binary logistic regression analysis was used. P 0.05 was chosen as the cutoff for statistical significance. If p 0.001, a highly significant difference was detected.

Ethical Consideration:

All procedures performed in the study were in accordance with the ethical standards of the faculty of medicine, Ain Shams University Research and ethical committee. We obtained approval from Research Ethics Committee (REC) No. FWA 000017585. FMASU R 160/2023. Written informed consent was obtained from participants for participation in this study.

The FMASU REC is organized and operated according to guidelines of the International Council on Harmonization (ICH) and the Islamic Organization of Medical Sciences (IOMS), the United States Office for Human Research Protections, and the United States Code of Feral Regulations and operates under Federal Wide Assurance No. FWA 000017585. FMASU R 160/2023.

RESULTS:

Of 50 RA patients, females were 92% of our patients. They were between the ages of

34 and 68 years with the disease persistence anywhere between one to twenty-seven years. Different biological therapy was used by 72% of our patients, together with other conventional therapies Table (1). The descriptive and laboratory data are explained in Tables (1 and 2).

Table 1: Descriptive data of the studied patients.

50 RA patients				
Sex		N		%
	Female	46		92
	Male	4		8
Age (Years)	Range	34	-	68
	Mean ± SD	51.214	±	8.317
DD (Years)	Range	1	-	27
	Mean ± SD	10.205	±	4.153
Medical treatment		N		%
	Non-Biologics	14		28
	Biologics	36		72
	Methotrexate	47		94
	Leflunomide	1		2
	Glucocorticoid <7.5mg	7		14
	Glucocorticoid >7.5mg	22		44
	Hydroquine	9		18
	Etanercept (Enbrel®)	12		24
	Adalimumab (Humira®)	4		8
	Adalimumab (Amgivita®)	10		20
	Golimumab (Simponi®)	10		20
ESR (mm/h)	Range	12	-	76
	Mean ± SD	40.67	±	23.18
DAS-ESR	Range	2.3	-	7.5
	Mean ± SD	4.1	±	1.32
		N		%
	Remission	9		18
	Low activity	5		10
	Moderate activity	26		52
	High activity	10		20

RA: Rheumatoid arthritis, DD: Disease duration, ESR: Erythrocyte sedimentation rate, DAS-ESR: Disease Activity Score using Erythrocyte sedimentation rate, SD: Standard of Deviation, N: Numbers, mm/h: millimeter per hour, mg: milligram.

Table 2: Laboratory data of the studied patients.

50 RA patients				
Hb (g/dl)	Range	10.17	-	14.3
	Mean ± SD	11.14	±	0.75
RBCs (million/mm^3)	Range	3.41	-	5.8
	Mean ± SD	4.66	±	0.5
WBCS (/mm^3)	Range	3.74	-	18.75
	Mean ± SD	7.39	±	2.94
PLT (/mm^3)	Range	130.42	-	658.51
	Mean ± SD	392.53	±	171.6

RA: Rheumatoid arthritis, WBCs: White blood cells, RBCs: Red blood cells, Hb: Hemoglobin, PLT: Platelets, /mm^3: Per cubic millimeter, %: percentage, g/dl: Gram per deciliter, fl: femtoliter, pg: picogram, ng/ml: nanogram per milliliter, µg/dl: microgram per deciliter, SD: Standard Deviation.

Based on the World Health Organization (WHO) criteria for anemia [9], 86% of our patients suffered from anemia particularly the microcytic hypochromic type (76%). Iron deficiency anemia was diagnosed in 22% and ACD in 54% of patients based on serum ferritin and serum iron levels Table (3).

Table 3: Anaemia profile of the studied patients

50 RA patients				
Anemia		N		%
	Anemic patients	43		86
	Microcytic hypochromic anemia	38		76
	ACD	27		54
	Iron deficiency anemia	11		22
Hb (g/dl)	Range	10.17	-	14.3
	Mean ± SD	11.14	±	0.75
HCT (%)	Range	30	-	42.3
	Mean ± SD	34.28	±	2.45
RBCs (million/mm^3)	Range	3.41	-	5.8
	Mean ± SD	4.66	±	0.5
MCV (fl)	Range	50	-	107
	Mean ± SD	78.92	±	7.41
MCH (pg)	Range	19.8	-	35
	Mean ± SD	25.54	±	3.27
RDW (%)	Range	11.2	-	19.1
	Mean ± SD	13.04	±	1.61
Ferritin (ng/ml)	Range	53.3	-	248.6
	Mean ± SD	134.6	±	79.4
Iron (µg/dl)	Range	15.5	-	96.2
	Mean ± SD	48.7	±	32.1

RA: Rheumatoid arthritis, ACD: Anemia of chronic disease, HCT: Hematocrit, RBCs: Red blood cells, Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular Hemoglobin, RDW: Red cell distribution width, /mm^3: Per cubic millimeter, %: percentage, g/dL: Gram per deciliter, fl: femtoliter, pg: picogram, ng/ml: nanogram per milliliter, µg/dl: microgram per deciliter, SD: Standard Deviation.

Our data showed that serum level of hepcidin ranged from 0.3 to 45.8 ng/ml (14.2±1.3). The anemic patients had significantly higher serum hepcidin concentrations than the non-anemic patients, with higher levels in the ACD patients Table (4).

Table 4: Comparison between the anemic and non-anemic patients.

	Anemic No. (43)		Non-anemic No. (7)	P-value
	ACD No. (27)	Non-ACD No. (16)		
	Mean ± SD		Mean ± SD	
Hb (g/dL)	11.1	10.8	13.5	<0.001**
HCT (%)	35.7	32.4	38.6	<0.001**
RBCs (million/mm^3)	4.5	4.2	5.1	0.03*
MCV (fl)	79.8	76.1	86.2	0.04*
MCH (pg)	25.7	24.6	32.7	0.06
RDW (%)	13.4	13.0	12.3	0.03*
Ferritin (ng/ml)	183.6	75.3	175.2	0,41
Iron (µg/dl)	69.3	32.2	91.6	0.007*
Hepcidin (ng/ml)	15.7	14.3	8.4	<0.001**
DAS-ESR	4.8	5.0	2.3	<0.001**

ACD: Anemia of chronic disease, HCT: Hematocrit, RBCs: Red blood cells, Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular Hemoglobin, RDW: Red cell distribution width, /mm^3: Per cubic millimeter, %: percentage, g/dl: Gram per deciliter, fl: femtoliter, pg: picogram, ng/ml: nanogram per milliliter, µg/dl: microgram per deciliter, SD: Standard Deviation, P: probability value, *: significant, **: highly significant.

According to the DAS-28 score using ESR, 41 of our patients (82%) were in an active state, while only 9 (18%) were inactive. The active patients had significantly higher serum hepcidin concentrations than the in-active patients Table (5).

Table 5: Comparison between active and inactive patients.

	Active No. (41)	In-active No. (9)	T-Test	
	Mean ± SD	Mean ± SD	T	P-value
ESR (mm/h)	49.78 ± 20.34	16.12 ± 2.7	6.773	<0.001**
DAS-ESR	4.8 ± 1.1	2.5 ± 0.1	2.134	0.0007**
Hepcidin (ng/ml)	15.3 ± 0.6	7.1 ± 2.02	6.305	<0.001**

ESR: Erythrocyte sedimentation rate, DAS-ESR: Disease Activity Score using Erythrocyte sedimentation rate, SD: Standard of Deviation, mm/h: millimeter per hour, ng/dl: nanogram per milliliter, t: independent sample t-test, P: probability value, **: highly significant.

There was a positive correlation between serum hepcidin, and the laboratory parameters used to detect the activity including ESR, and DAS-28. On the other hand, we observed a negative correlation between serum hepcidin and Hb, MCH, and serum iron. No significant correlation could be seen with RBC, WBC, or platelet counts Table (6).

Table (6): Correlation between serum Hepcidin and different parameters

	Serum Hepcidin	
	R	P
ESR	0.563	<0.001**
DAS-ESR	0.578	<0.001**
RBC count	0.154	> 0.05
WBC count	0.283	> 0.05
Platelet Count	0.206	> 0.05
Hb	-0.548	<0.001**
HCT	-0.094	> 0.05
MCV	0.252	> 0.05
MCH	-0.601	<0.001**
RDW	0.417	> 0.05
Ferritin	0.341	> 0.05
Serum Iron	-0.367	<0.01*

ESR: Erythrocyte sedimentation rate, DAS-ESR: Disease Activity Score using Erythrocyte sedimentation rate, HCT: Hematocrit, RBCs: Red blood cells, WBCs: White blood cells, Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular Hemoglobin, RDW: Red cell distribution width, P: probability value, **: highly significant, *: Significant.

DISCUSSION:

With irreversible cartilage and joint damage, rheumatoid arthritis is the most prevalent chronic autoimmune inflammatory arthritis [(10)]. Hepcidin is an acute-phase reactant protein associated with two main conditions acting as a homeostatic regulator of iron metabolism and an inflammatory mediator [(11)]. One of the common comorbidities in RA patients is anemia. RA can be associated with different types of anemia, including anemia of chronic disease (ACD) and iron deficiency anemia [(12)]. Many researchers concluded that RA patients with anemia and with elevated serum ferritin levels (> 50 g/l)**,** excluding iron deficiency, will have an ACD [(12)]**.** In the current study, we evaluated the serum hepcidin level in patients with RA and its correlation with hematological profile and disease activity.

Most of our patients (86%) were diagnosed with anemia according to WHO diagnostic criteria for anemia [(9)], where most of them (76%) had the microcytic hypochromic type. Iron deficiency anemia

was diagnosed in 22% while ACD in 54% of patients based on the serum ferritin and serum iron levels. This was ongoing with almost all studies that define ACD as the most frequent cause of anemia associated with RA [5,12-14]. ACD in RA has multifactorial causes, including ineffective erythropoiesis, abnormal iron metabolism, and increased inflammatory markers such as IL-6 and TNF-α [5]. This could explain why ACD is associated with higher disease activity and also the fact that the frequency and intensity of all types of anemia in active RA patients are higher than that in inactive patients. High disease activity in most of our recruited patients explains the high percentage of anemic patients in this research. ACD is usually mild anemia characterized by decreased serum iron, total iron-binding capacity, iron saturation, bone marrow sideroblast, and normal or increased reticuloendothelial iron [15].

In this study, the anemic patients had significantly higher serum hepcidin concentrations than the non-anemic patients, with higher levels in the ACD patients. This increased level was reported in much other research that observed the same increase in hepcidin level [6&14]. This is due to the chronic inflammatory status in RA that results in increased circulating pro-inflammatory cytokines which influence the production and function of hepcidin with a direct effect on the serum iron levels and ferritin [5]. According to the anaemic profile, significant correlations were observed between hepcidin and Hb and serum ferritin. We found that hepcidin has a strong correlation with iron-deficiency anemia and iron metabolism in specific not anemia in general. Similar results were reported by many other studies where researchers found that hepcidin acts as a signalling molecule that affects in different ways the preservation of iron function and homeostasis [16-18]. A study conducted by Deicher and HÖrl concluded that the acute inflammation provoked by a single turpentine injection can cause a two-fold reduction in serum iron levels in wild-type mice [19]. These findings imply that hepcidin is crucial for maintaining iron homeostasis when inflammation is active and ongoing.

Despite our finding that the serum hepcidin in our patients suffering from ACD was higher than those with non-ACD, the difference was not statistically significant. This could conclude that when inflammation and anemia coexist, inflammation regulates the expression of hepcidin. The iron levels in our patients were negatively correlated with their hepcidin levels, which supports earlier claims. Yet, this was supported by many other previous studies where they supported the same conclusion [6&11&14]. There is an agreement that the relation between the degrees of inflammation, extent of anemia, and serum level of hepcidin is complex and multifactorial. There are many scopes for the anemia occurring in RA, including the direct effect of TNF alpha and IL1 on bone marrow causing its suppressive action on RBC production also the effect of TNF alpha and interferon-gamma on renal erythropoietin production. Moreover, the production of hepcidin, increased by IL6 through STAT3 inducing gene expression, downregulates intestinal iron absorption and prevents its release from the reticuloendothelial system [18]. Consequently, causing aggravation of anemia even in the presence of adequate iron storage. Adding to this the local GIT causes that may affect serum iron levels as bleeding and impaired absorption. Accordantly, serum hepcidin can't be of value to differentiate between ACD and non-ACD due to overlapping causes of anemia and the elevated serum hepcidin level due to RA pathological process regardless of the type of accompanying anemia.

Finally, our study showed a highly significant correlation between the serum level of hepcidin, and the extent of disease activity as measured by the DAS-28 activity score where we used the version that combines the ESR level. Hepcidin acts as an

acute-phase reactant produced in the liver hepatocytes that displays intrinsic antimicrobial activity [20]. Being induced by IL-6, hepcidin production in the liver has been linked to the increase in RA disease activity as IL6 acts as a main multifunctional cytokine in joint destruction and progression of the inflammatory process [21]**.** The focus on the serum hepcidin level as a marker that might be used to assess the activity of RA has mostly been due to the correlation found between IL-6 as a cornerstone cytokine in RA disease induction, activity, joint destruction, and ACD mediator as well as it induces the hepcidin production [21-23]. This also may explain the fact of improvement that occurs in the parameters of anemia accompanying the use and the response to anti-TNF treatments. In addition, a study conducted by *Østgård and his colleagues in 2017*, where they measured plasma hepcidin levels in eighty RA patients at the beginning of the study and repeated after receiving treatment using DMARDs and anti-TNF for 52 weeks, this was followed by reassessment. They noticed that there was a corresponding drop in serum hepcidin levels with the reduction in disease activity [24]. Our results were consistent with those obtained by many other similar researchers, where *Kim and his colleagues* measured serum concentration of pro hepcidin in 40 RA patients concluding that it was significantly correlated to the extent of disease activity and could be a detector of activity [25]. Also, Menha and their colleagues performed their study on 185 RA patients relating their disease activity to serum hepcidin and interleukin-1 receptor antagonist gene concluding similar results [26]**.** On the other hand, some studies observed a lack of correlation between serum hepcidin and RA disease activity [6&27]**.** This could be attributed to the fact that the design of these studies and their findings interpretation were different from ours although they documented that serum hepcidin is higher in erosive active RA, they doubted the role of hepcidin in chronic inflammation which might be reflected on its serum level. The study done by Sahebari and their colleagues [6] was done for 80 RA patients where they excluded patients with anemias for any other cause than ACD also all of their patients were on methotrexate therapy combined with oral steroids, while 80% of our patients were on biological therapy which may explain the discrepancy in the blood profiles and the disease outcomes.

It should be noted that while the analytical variation tends to be low or consistent across all procedures, the serum hepcidin levels as assessed by different methods may differ significantly [28]. There are some difficulties in determining the serum level of hepcidin in rheumatoid patients including age-matched groups, duration of the disease, and different types of treatments received by the patients. In addition to these difficulties, hepcidin tends to form aggregates and cling to surfaces, resulting in some technical challenges with the techniques used to determine its level in clinical research [16]. On the other hand, this peptide gained attention in the follow-up of patients with RA and to determine the clinical efficacy of some therapeutic interventions as in the study performed by *Doyle and his colleagues* where they evaluated the effect of anti-TNF treatment on RA patients with anemia concluding that hepcidin was one the inflammatory markers that correlated with improvement of activity with the disease control [29]**.** Also, another study done by *Abu-Zaid et al.* conducted on 80 RA patients evaluated the effect of Etanercept and adalimumab on anemia and hepcidin levels, they observed that the treatment improved ACD with a significant decrease of hepcidin level with the improvement of disease activity [30]**.** However, more research are needed to offer additional data before applying this protein as a routine tool in the clinical management of this disease [6]**.**

There are some limitations regarding our research as the absence of bone marrow

examination for accurate diagnosis of iron deficiency, the small number of participants for each study sub-group, limited patients' previous laboratory data, and unavailable follow-up assessment.

Conclusion:

We concluded from our study that serum hepcidin could be of value in assessing the degree of disease activity in rheumatoid arthritis patients and determining the extent of rheumatoid-related anemias as well.

Declarations:

Consent for publication:

Not applicable due to patients' privacy concerns.

Availability of data and materials:

The datasets generated and/or analyzed during this study are not publicly available due to patients' privacy but are available from the corresponding author upon reasonable request.

Conflicts of interests:

The authors declare that they have no competing interests concerning this article.

Funding:

The authors received no specific funding for this work.

Authors contributions:

ShM, AM, SM: recruited patients, carried out clinical examination and assessment, blood sample aspiration and collection, and all contributed to writing, editing, and revising the manuscript.

MM: underwent data tabulation and statistical analysis, and interpreted the patient's data, wrote the results and contributed to writing and editing the manuscript.

HS: designed the protocol, carried out the Ethical approval, and data collection, and underwent all the laboratory analysis.

All authors have agreed to the conditions noted on the Authorship Agreement Form and have read and approved the final version submitted. The manuscript's content has not been published or submitted for publication elsewhere. All authors read and approved the final manuscript.

The corresponding author accepts responsibility for all stated information contained within it.

Acknowledgments:

We appreciate all participants in this research. This research was done in the Physical medicine Clinic, Ain Shams University and Clinic of Internal Medicine.

REFERENCES:

1. **Duffield SJ, Miller N, Zhao S, Goodson NJ**. Concomitant fibromyalgia complicating chronic inflammatory arthritis: a systematic review and meta-analysis. Rheumatology (Oxford). 2018; 57(8):1453–60.
2. **Larsson I.** Patients' conceptions of their own influence on good treatment response to biological therapy in chronic inflammatory arthritis. Patient Prefer Adherence. 2017; 11:1057–67.
3. **Weiss G, Goodnough LT**. Anemia of chronic disease. N Engl J Med. 2005; 352:1011-23.
4. **Means RT**. Hepcidin and anemia. Blood Rev. 2004; 18:219–25.
5. **Masson C**. Rheumatoid anemia. Joint Bone Spine. 2011; 78(2):131-7.
6. **Sahebari M, Rezaieyazdi Z, Hashemy SI, Khorasani S, Shahgordi S, Alizadeh MK, Ghaeni A, Khodashahi M.** Serum hepcidin level and rheumatoid arthritis disease activity. Eur J Rheumatol. 2018; 6(2):76-80.
7. **Aletaha D, Neogi T, Silman AJ, Funovits JL, Felson DT, Bingham CO, et al**. Rheumatoid arthritis classification criteria: An American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Arthritis. Rheum. 2010; 62 (9): 2569-81.

8. **Prevoo MLL, Van'T Hof MA, Kuper HH, Van Leeuwen MA, Van De Putte LBA, and Van Riel PLCM.** Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis: MODIFIED DISEASE ACTIVITY SCORE. Arthritis Rheum. 1995; 38(1):44–8.
9. **Nutritional anemias.** Report of a WHO scientific group. World Health Organ Tech Rep Ser. 1968; 405:5-37. PMID: 4975372.
10. **Melchiorre D, Iagnocco AM, Acharya S, Pepe G, Borderi M.** Rheumatoid arthritis. Autoimmune Dis. 2013; 2013:958437. doi: 10.1155/2013/958437. Epub 2013 Nov 28. PMID: 24369497; PMCID: PMC3863497.
11. **Geerts I, Vermeersch P, Joosten E.** Evaluation of the first commercial hepcidin ELISA for the differential diagnosis of anemia of chronic disease and iron deficiency anemia in hospitalized geriatric patients. ISRN Hematol. 2012; 2012:567491. doi: 10.5402/2012/567491. Epub 2012 Feb 29. PMID: 22461996; PMCID: PMC3302103.
12. **Swaak**. Anemia of Chronic Disease in Patients with Rheumatoid Arthritis: Aspects of Prevalence, Outcome, Diagnosis, and the Effect of Treatment on Disease Activity. The Journal of Rheumatology. 2006; 33:8.
13. **Peeters HRM, Jongen-Lavrencic M, Raja AN, et al.** Course and characteristics of anemia in patients with rheumatoid arthritis of recent onset. Ann Rheum Dis. 1996; 55:162-8.
14. **Demirag MD, Haznedaroglu S, Sancak B, Konca C, Gulbahar O, Ozturk MA, et al**. Circulating hepcidin in the crossroads of anemia and inflammation associated with rheumatoid arthritis. Intern Med. 2009; 48:421-6.
15. **Cartwright GE, Lee GR**. The anemia of chronic disorders. Br J Haematol. 1971; 21:147-52.
16. **Sabău A., Văleanu M., Boloşiu H., Crăciun A.** Evaluation of serum hepcidin variation in patients with rheumatoid arthritis according to anemia profile and its correlation with disease activity. Revista Romana de Medicina de Laborator. 2013; 21(1): 17-27. https://doi.org/10.2478/rrlm-2013-0014
17. **Sato H, Takai Ch, Kazama JJ, Wakamatsu A, Hasegawa E, Kobayashi D, Kondo N, Nakatsue T, et al.** Serum hepcidin level, iron metabolism and osteoporosis in patients with rheumatoid arthritis. Scientific Reports. 2020; 10:9882.
18. **Tański W., Chabowski M., Jankowska-Polańska B., Jankowska E.** Anaemia and iron deficiency in patients with rheumatoid arthritis and other chronic diseases*. Postępy Higieny i Medycyny Doświadczalnej. 2021; 75(1): 143-151. https://doi.org/10.5604/01.3001.0014.7838
19. **Deicher R, HÖrl WH**. Hepcidin: a molecular link between inflammation and anemia. Nephrol Dial Trans-plant. 2004; 19(3):521-4.
20. **Malyszko J, Malyszko JS, Pawlak K, Mysliwiec M**. Hepcidin, an acute-phase protein and a marker of inflammation in kidney transplant recipients with and without coronary artery disease. Transplant Proc. 2006; 38(9):2895-8.
21. **Rodriguez R, Jung CL, Gabayan V, Deng JC, Ganz T, Nemeth E, et al**. Hepcidin induction by pathogens and pathogen-derived molecules is strongly dependent on interleukin-6. Infect Immun. 2014; 82:745-52.
22. **Hashizume M, Mihara M.** The roles of interleukin-6 in the pathogenesis of rheumatoid arthritis. Arthritis. 2011; 2011:765624. doi: 10.1155/2011/765624. Epub 2011 May 26. PMID: 22046525; PMCID: PMC3199948.
23. **Raj DS**. Role of interleukin-6 in the anemia of chronic disease. Semin Arthritis Rheum. 2009; 38:382-8.
24. **Østgård RD, Glerup H, Jurik AG, Kragstrup TW, Stengaard-Pedersen K, Hetland ML, Hørslev-Petersen K, Junker P, Deleuran BW**. Hepcidin plasma levels are not associated with changes in hemoglobin in early rheumatoid arthritis patients. Scand J Rheumatol. 2017; 46(6):441-5.
25. **Kim HR, Kim KW, Yoon SY, Kim SH, Lee SH**. Serum pro-hepcidin could reflect disease activity in patients with rheumatoid arthritis. J Korean Med Sci. 2010; 25:348-52.
26. **Swellam M, Gabal KM, Youssef SS**. Interleukin-1 receptor antagonist gene

polymorphism and hepcidin in rheumatoid arthritis: Correlations with clinical and laboratory indices of disease activity. IUBMB Life. 2013; 65:883-8.

27. **Sellam J, Kotti S, Fellahi S, Bastard JP, Meyer M, Liote F, et al**. Serum hepcidin level is not an independent surrogate biomarker of disease activity or of radiographic progression in rheumatoid arthritis: results from the ESPOIR cohort. Ann Rheum Dis. 2013; 72:312-4.
28. **Kroot JJC, Kemna EHJM, Bansal SS, Busbridge M, Campostrini N, Girelli D, et al.** Results of the first international round robin for the quantification of urinary and plasma hepcidin assays: need for standardization. Hematological. 2009; 94:1748-52.
29. **Doyle MK, Rahman MU, Frederick B, Birbara CA, de Vries D, Toedter G, et al.** Effects of subcutaneous and intravenous golimumab on inflammatory biomarkers in patients with rheumatoid arthritis: results of a phase 1, randomized, open-label trial. Rheumatology (Oxford). 2013; 52:1214-9.
30. **Abu-Zaid MH, El morsy S, Rageh EM, Shahba A, Gaber RA:** The effectiveness of etanercept and adalimumab on anemia of chronic disease and serum hepcidin in patients with rheumatoid arthritis, a comparative study. The Egyptian Rheumatologist. 2018; 40: 233-237. https://doi.org/10.1016/j.ejr.2017.09.004.

دراسة لمستوى الهيبسيدين في الدم و علاقته بمؤشرات النشاط الإكلينيكي ومعايير صورة الدم الكاملة في مرضى التهاب المفاصل الروماتويدي

شريهان مهدي سلامه[١]، ساميه محمد رشاد[١]، أيه محمد محمود الجندى[٢]، هبه سامى عجمى[٣] و منى محمد البابلى[١]

قسم الطب الطبيعي والروماتيزم والتأهيل بكلية الطب جامعة عين شمس١
قسم الباطنه العام - بكلية الطب جامعة عين شمس٢
قسم الباثولوجيه الاكلينيكيه - بكلية الطب جامعة عين شمس٣

خلفية البحث : التهاب المفاصل الروماتويدي هو التهاب مفاصل التهابي مزمن يصيب المناعة الذاتية ويصيب ٠,٥ إلى ١٪ من السكان. أحد الأمراض المصاحبة الشائعة التي يواجهها مرضى الروماتويد هو فقر الدم. يعتبر الهيبسيدين أحد البروتينات المتفاعلة أثناء الطور الحاد للمرض، فهو يعمل كمنظم استقلابي لاستقلاب الحديد وكوسيط للالتهابات أيضًا.

الهدف: تهدف هذه الدراسة إلى تقييم قيمة قياس مستويات الهيبسيدين في الدم لدى مرضى التهاب المفاصل الروماتويدي وعلاقته بنشاط المرض السريري ومؤشرات الدم.

طريقة البحث: خضع خمسون مريضًا من مرضى التهاب المفاصل الروماتويدي لتقييم طبي كامل مع تقييم درجة شدية المرض، والاختبارات المعملية بما في ذلك صورة دم كاملة، سرعة الترسيب، عامل الروماتويد، قياس مستوى الفيريتين والحديد بالدم، ومستوى الهيبسيدين في الدم.

النتائج: أظهرت نتائجنا أن هناك علاقة ذات دلالة إحصائية بين علامات الالتهاب ومستوى الهيبسيدين في الدم، وكذلك وجود علاقة إيجابية بين درجة نشاط المرض ومستوى الهيبسيدين في الدم. **الاستنتاج:** استنتجنا من دراستنا أن الهيبسيدين في الدم يمكن استخدامه لتقييم نشاط المرض لدى مرضى التهاب المفاصل الروماتويدي وفقر الدم المرتبط بالروماتويد أيضًا.