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Research Article

Serum Hepcidin: An Atherosclerotic Biomarker in Rheumatoid Arthritis Patients: A multicenter Case-Control Study

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Abstract

Objectives: Hepcidin, a major regulator of iron metabolism and homeostasis. Studies on hepcidin in rheumatoid arthritis (RA) are still inconsistent. Aim to identify if hepcidin could add to diagnosis of RA associated complications, especially atherosclerosis.

Methods: A case-control study involved measurement of serum hepcidin levels in patients and control by Enzyme-linked immunosorbent assay, and assessment of Carotid intima Media thickness (CIMT) by Doppler ultrasonography. Patients' clinical manifestations, comorbidities, and treatment were recorded. The disease activity score-28 (DAS28) was used to evaluate RA activity.

Results: This study enrolled 50 RA patients and 25 control subjects, with predominantly female participants (98%). Hepcidin is significantly increased in RA patients when compared to control ($p<0.001$). Right and Left Carotid intima media thickness showed significant difference between patients and control ($p=0.016$ & $p=0.006$). In multivariate linear regression models hepcidin showed significant positive correlation with BMI, CRP, CIMT ($p<0.001$). Hepcidin level ≥298.5 showed significant AUC that could discriminate between patients.

Conclusion: Increased hepcidin level in RA patients was correlated with CIMT ($r=0.676$). Patient Age, RA duration, cholesterol level and hepcidin level were linked to an increase in CIMT in RA patients. Therefore, serum hepcidin level could be a predictor of atherosclerosis in RA patients.

Keywords: Atherosclerosis, CIMT, Hepcidin, Rheumatoid arthritis, Rheumatoid factor

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Rheumatoid arthritis is a chronic immune mediated illness with no identifiable etiology. Joint inflammation and extra-articular involvement are the key features of RA. It is characterized by long-term inflammation, gradual joint degeneration, and increased morbidity. The prevalence rate is about 1% with about 3 out of every 10,000 people worldwide have RA, and the prevalence rate rises with age whereas the highest range is 35 to 50 years old. Men are three times less likely to get RA than females, although the gender gap narrows as people age an estimated preva-

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lence ranging from 0.4 to 1.1% globally and 0.3% in the Egyptian population. Main risk factors for RA include genetic predisposition accounting for 60% of cases and female gender where women are two to three times more likely to develop RA compared to men.[1]

RA individuals have more cardiovascular complications, and the mortality rates are relatively higher than the normal population. The relative risk of cardiovascular disease (CVD) has been reported to be between 1.5 and 4.0. The specific link mechanism between RA and CVD is yet fully recognized; this risk is suggested by chronic inflammation, which is likely the cause of premature atherosclerosis in RA whereas there are evident similarities between atherosclerotic plaque and synovitis in RA at histopathology view.[2]

Hepcidin is a peptide hormone produced in the liver that plays a crucial role in iron homeostasis and its production is encoded by the hepcidin antimicrobial peptide (HAMP) gene. It is a key regulator of the entry of iron into the circulation, hepcidin level is found to be abnormally high during inflammatory states; the increased blood hepcidin might be associated with the incidence and promotion of atherosclerosis, Hepcidin could be a potential biomarker for risk prediction in acute coronary syndrome patients.[3]

Our study aimed to identify the correlation between serum hepcidin level and carotid intimal medial thickness (CIMT) in Rheumatoid Arthritis patients.

Methods

A multicenter case-control study was conducted on RA patients attending the rheumatology and immunology outpatients' clinics and inpatients wards, Menoufia University Hospital and Benha teaching hospital, from January 2021 to March 2023. The study participants were divided into two groups, Group 1, including 50 Rheumatoid adult patients and Group II 25 subjects as control group. Both groups were matched in age, sex, BMI and comorbidities. Patients with RA were classified as such by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) according to ACR/EULAR criteria.[4] RA classification categories range from 0 to 10 according to the implemented criteria. If a patient has a point total of six or more and has synovitis in at least one joint and no other diagnosis better explains the synovitis, they are categorized as having RA.

Patients were on conventional disease modifying antirheumatic drugs, none of these patients were on biological treatment especially tocilizumab, nor JAK2 inhibitor which could affect the hepcidin level.[5,6] RA patients on tocilizumab or JAK inhibitors were excluded from the study, the exclusion extended to patients with systemic lupus erythematosus, osteoarthritis, scleroderma, antiphospholipid syndrome, or any other connective tissue disease rather than RA. Patients on lipid-lowering medications, adult females older than 65 and younger than 18 and smoker patients were also excluded from the study.

All the participants were asked about their medical history and underwent a complete physical examination. Weight/height2 (kg/m^2) was used to determine the body mass index (BMI), an underweight BMI is less than 18.5 kg/m^2, a healthy BMI is between 18.5-24.9, an overweight BMI is 25-29.9, and an obese BMI is 30 or more. Complete Blood count, Lipid profile (Total Cholesterol, Triglycerides, HDL-Cholesterol and LDL-cholesterol by enzymatic colorimetric method, fasting and two hours postprandial blood glucose and HbA1C, Rheumatoid Factor (RF), Anti-Cyclic citrullinated peptide (Anti- CCP), Antinuclear Antibody (ANA) (line blot techniques detected ANA), Erythrocyte Sedimentation Rate (ESR), and C-Reactive Protein (CRP). A positive result was CRP levels of more than 8 mg/L. Enzyme linked immunosorbent assay (ELISA) was used in measurement of serum hepcidin.

The distance between the leading margins of the first and second echogenic lines on both sides of the far walls of the common carotid artery's distal segment and its bifurcation and internal carotid artery was measured using Doppler ultrasonography (GE logic E10 USA) and a duplex ultrasound system operating in B-mode at 7.5 MHz scanning frequency. Measurements were conducted at intervals of 0.5, 1, and 2 cm below and above the bifurcation point, respectively. It took an average of three measures to come up with each result, and just one person was involved in the measuring process. The statistical comparison of CIMT thickness between patients and control groups was carried out using the mean values.

Regarding ethical approval, The Menoufia Faculty of Medicine's local ethics committee gave its approval on 10/18 INTM. According to the recommendations of the local ethics committee, all participants gave their informed permission to participate in the study.

Statistical Analysis

The data was processed using an IBM-compatible personal computer with the Statistical Package for the Social Sciences version 26 (SPSS Inc., 2015). Number (N) and percentage (%) were used to describe qualitative data. Median and interquartile ranges (IQR) were used to express quantitative data. Furthermore, quantitative data were classified using the mean for data that is normally distributed and the median for data that is not normally distributed. The

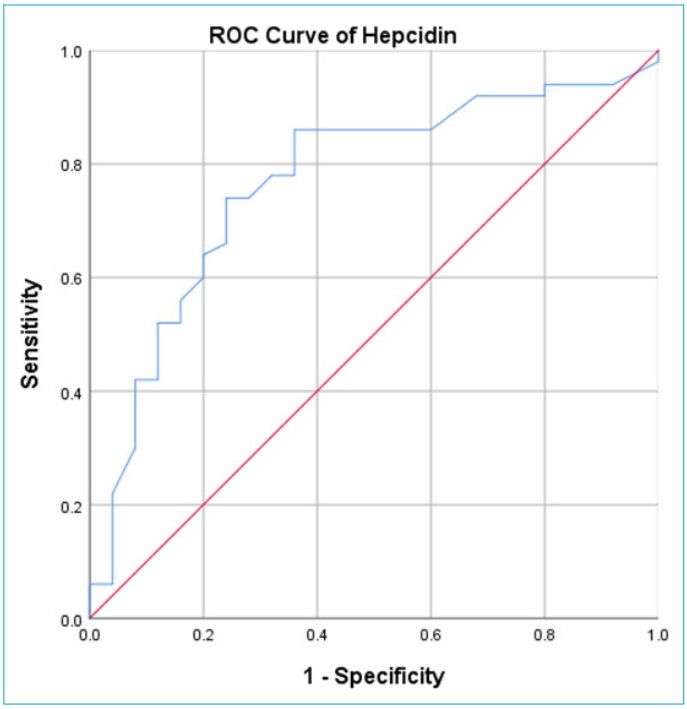

Figure 1. Receiver operating characteristic curve analysis of the optimal cutoff of Hepcidin levels.

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to determine the normality of a distribution. For comparing quantitative variables between two groups of normally distributed data, the student's t-test (t) was used.

In contrast, the Mann-test Whitney's (U) was used to compare quantitative variables between two groups of not normally distributed data. The Chi-square test was used to investigate the relationship between qualitative variables. Receiver Operator Characteristic curves with the Area Under the Curve were used to determine the optimal cutoff. Spearman rank correlation coefficient was used to correlate 2 non-parametric variables. To investigate risk factors for increased CIMT, univariate and multivariate logistic regression analyses were performed. Factors with a $p<0.05$ in univariate analysis were included in multivariate analysis. The results were expressed as an adjusted odds ratio (OR) with a 95% confidence interval (CI). A significant difference is considered when $p<0.05$.

Results

The mean age of the studied 50 RA patients was 47.8±10.73, with female predominance 98%, most cases had no comorbidities, and the most common associated comorbidity was diabetes. All cases had positive RF/ Anti CCP. Median CRP was 20, ESR was 38, TG 92, Cholesterol was 152, LDL 97.5, HDL 59.8, hepcidin was 326.9±49.11, Right CIMT was 0.65, and Left CIMT was 0.6 (Table 1).

Table 1. Demographic, clinical and laboratory characteristics of RA patients (n=50)

Variable	Patients (n=50) No (%)
Age (years) (Mean±SD)	47.8±10.73
Sex	
Male	1 (2)
Female	49 (98)
BMI (Mean±SD)	30.5±3.75
Family history of premature atherosclerosis	
Yes	7 (14)
Comorbidities	
None	31 (62)
Diabetes	8 (16)
DM&HTN	4 (8)
CVS	7 (14)
Disease duration (years) (Mean±SD)	8.38±6.2
DAS (Mean±SD)	3.7±0.97
Extra articular manifestations	
Positive	10 (31)
Negative	40 (80)
RF/Anti ccp	
Positive	50 (100)
Negative	0 (0.0)
CRP (mg/L) Median (IQR)	20 (9.75 -33)
ESR (mm/hr), Median (IQR)	38 (25-55)
TG (mg/dl), Median (IQR)	92 (61.25-129.5)
Cholesterol (mg/dl) Median (IQR)	152 (98.75–188.5)
LDL (mg/dl) Median (IQR)	97.5 (83–126.25)
HDL (mg/dl) Median (IQR)	59.8 (39.75–77.75)
Right CIMT (mm) Median (IQR)	0.65 (0.5-0.83)
Left CIMT (mm) Median (IQR)	0.6 (0.6-0.8)
Hepcidin	326.9±49.11

SD: standard deviation; range: minimum-maximum; No: number, %: percentage; IQR=inter quartile range (25-75); DAS: Disease Activity Score; CRP: C - reactive protein; ESR: erythrocyte sedimentation rate; TG: triglyceride; HDL: high density lipoprotein; CIMT: Carotid intima-media thickness.

The cases group had substantial increases in family history, CRP, ESR, TG, Cholesterol, and LDL compared to the control group. Hepcidin levels in the RA group were considerably greater than in the control group. Right and left CIMT were found to vary significantly between the cases and the control group. No significant differences were detected across the study groups in terms of age, sex, BMI, comorbidities, and HDL ($p>0.05$) (Table 2).

Right CIMT and Left CIMT showed a significant positive correlation with age, disease duration, CRP, TG, cholesterol and hepcidin level. While Left CIMT alone indicated a very substantial positive connection with LDL (Table 3).

Table 2. Comparing demographic, clinical, and laboratory characteristics between RA patients and controls

Variable	Patients (n=50) No. (%)	Controls (n=25) No. (%)	Test of significance	p
Age (years): (Mean±SD)	47.8±10.73	42.36±12.6	t=1.954	0.055
Sex				
Male	1 (2)	3 (12)	χ^2=3.301	0.069
Female	49 (98)	22 (88)		
BMI kg/m^2 (Mean±SD)	30.5±3.75	29.1±3.75	t=1.597	0.115
Family history				
Yes	7(14)	0(0.0)	χ^2=3.860	0.049*
Comorbidities				
None	31 (62)	18 (31)	χ^2=2.285	0.515
Diabetes	8 (16)	3 (12)		
DM&HTN	4 (8)	3 (12)		
CVS	7 (14)	1 (4)		
RF/Anti ccp				
Positive	50 (100)	0 (0.0)	χ^2=75	<0.001*
Negative	0 (0.0)	25 (100)		
CRP (mg/L)				
Median (IQR)	20 (9.75 -33)	2 (2 -12.5)	U=3.927	<0.001*
ESR (mm/h)				
Median (IQR)	38 (25-55)	17 (9-21)	U=5.319	<0.001*
TG (mg/dl)				
Median (IQR)	92 (61.25-129.5)	107 (85.5-117.5)	U=2.4	0.016*
Cholesterol (mg/dl)				
Median (IQR)	152 (98.75-188.5)	105 (95–115.5)	U=3.097	0.002*
LDL (mg/dl)				
Median (IQR)	97.5 (83 – 126.25)	85 (77.5 –90)	U=2.434	0.015*
HDL (mg/dl)				
Median (IQR)	59.8 (39.75 –77.75)	67.3 (62- 87.5)	U=1.079	0.280
Right CIMT (mm)				
Median (IQR)	0.65 (0.5 -0.83)	0.6 (0.5-0.7)	U=2.399	0.016*
Left CIMT (mm)				
Median (IQR)	0.6 (0.6-0.8)	0.6 (0.5-0.7)	U=2.75	0.006*
Hepcidin (pg/ml)				
(Mean±SD)	326.92±49.11	281.77±38.32	t=4.334	<0.001*

SD: standard deviation; Range: minimum-maximum; No: number; %: percentage; *: statistically significant. U= Mann-Whitney χ^2=Chi square; t=student t-test; DAS: Disease Activity Score CRP; C - reactive protein; ESR: erythrocyte sedimentation rate; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; CIMT: Carotid intima-media thickness; BMI: body mass index.

Multivariate analysis was conducted for prediction of factors associated with increased CIMT in rheumatoid patients. CRP, cholesterol level, ferritin and hepcidin level were significant predictors (Table 4).

Receiver operating characteristic curve analysis of the optimal cutoff of CIMT and Hepcidin levels. Hepcidin level ≥298.5 showed significant AUC that could discriminate between patients and control with sensitivity 72%, specificity 76%. CIMT ≥ 0.55 showed significant AUC that could discriminate between patients and control with sensitivity 82%, specificity 48% (Table 5).

Hepcidin level shows significant positive correlation with BMI, CRP, disease duration, cholesterol, ferritin, right CIMT and left CIMT. No significant correlation was found between hepcidin and age, ESR, LDL, HDL, Iron (ug/dl), TIBC and Transferrin saturation (Fig. 1).

Table 3. Correlation between carotid artery intima media thickness (CIMT) & other variables of the RA patients (n=50)

Variable	Right CIMT		Left CIMT	
	Rho	p	rho	p
Age (years)	0.359	0.011*	0.310	0.029*
BMI	0.215	0.134	0.281	0.148
Disease duration	0.386	0.006*	0.375	0.002*
ESR	0.082	0.572	0.078	0.558
CRP	0.214	0.04*	0.4	0.004*
TG	0.327	0.02*	0.316	0.025*
Cholesterol	0.412	0.003*	0.507	<0.001*
LDL	0.233	0.06	0.259	0.04*
HDL	-0.135	0.349	-0.118	0.182
Hepcidin	0.676	<0.001**	0.669	<0.001**

*: statistically significant; rho =spearman's rank correlation coefficient; CRP: C - reactive protein; ESR: erythrocyte sedimentation rate; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; BMI: body mass index.

Discussion

Rheumatoid arthritis is a chronic, progressive, systemic inflammatory disease with few numbers of studies have investigated the association of serum hepcidin levels in RA and its extra articular complications, whereas the results were inconsistent. Main risk factors for RA include genetic predisposition accounting for 60% of cases and female gender where women are two to three times more likely to develop RA compared to men.[7]

The present study showed that the mean age of the studied cases was 47.8±10.73 with Female predominance (98%), this agreed with Garrigues et al., who reported that 29 (72%) were female, mean age in their study was 55.9 years (SD: 14) and mean disease duration was 11.2 years (SD: 8.7).[8] Numerous factors have been implicated in the overrepresentation of women in RA and other autoimmune diseases, including hormonal, genetic, lifestyle, and environmental factors.[9]

The present study showed that most cases had less comorbidities, and the most associated comorbidity was diabetes, diabetes with hypertension followed by cardiovascular disease. Kłodziński and Wisłowska demonstrated that the most common comorbidities in their RA patients were hypertension and osteoporosis or osteopenia.[10]

The present study revealed that all cases had positive RF/Anti ccp. Median CRP was 20, ESR was 38, CRP, ESR showed significant increase in cases group when compared to control group. Previous study by Dessie et al., found that Mean±SD of hsCRP level among cases and control groups were 10.54±17.26 and 3.54±7.60, respectively.[9] Also, Naredo et al found that RF was positive in 30 (71.4%) patients and negative in 12 (28.6%) patients. The mean±SD positive RF value was 135±160 IU/ml (range 16–880 IU/ml).[12]

Furthermore, the present study reported that TG, cholesterol, LDL were significantly increased in RA patients when compared to control group. However, TG showed a significant decrease in cases when compared to the control group. Contrarily to our results, Mohan et al., who compared lipid profile among patients with RA and normal controls, there was no significant difference in the mean serum cholesterol; TG; LDL; and HDL levels between the two groups.[13]

In the present study serum hepcidin levels were significantly increased in RA patients when compared to the controls. This may be due to the high levels of IL-6, which is the main inducer of hepcidin and contributes to the development of RA. In line with our findings, previous studies reported that serum hepcidin levels were higher in RA patients compared to healthy controls.[14-17]

In the contrary to our results, Teke et al.,[18] reported that, there were no significant difference in serum hepcidin levels between their RA patients and healthy controls, which may be related to the hypoxia, despite in an inflammatory setting. In addition, studies upon the relationship between

Table 4. Multivariate logistic regression analysis for variables associated with increasing CIMT in rheumatoid patients.

Predictors (Independent variables)	B coefficient	Multivariate logistic regression		
		Odds Ratio	p	95% CI (lower-upper)
BMI (kg/m^2) (≥30)	1.422	4.147	0.114	0.711 – 24.181
Duration of disease (≥8 years)	1.281	3.599	0.097	0.795 – 16.295
CRP (mg/l) (≥20)	1.511	4.533	0.037*	1.099 – 18.704
Cholesterol (mg/dl) (≥152)	1.801	6.053	0.045*	1.009 -36.3
Ferritin (ng/ml) (≥80)	0.920	2.508	0.360	0.350 – 17.975
Hepcidin (pg/ml) (≥326)	1.905	6.718	0.03*	1.197 – 37.710

CI= Confidence interval; *: statistically significant; # Using the risky level to predict increasing; CRP; C - reactive protein; BMI: body mass index; CIMT: Carotid intima media thickness.

Table 5. Correlation between Hepcidin level & other variables of RA patients (n=50)

Variable	Hepcidin level	
	rs	p
Age (years)	0.2	0.165
BMI	0.368	<0.001**
Disease duration (years)	0.291	0.04*
ESR	0.02	0.888
CRP	0.516	<0.001**
TG	0.232	0.104
Cholesterol	0.301	0.034*
LDL	0.021	0.886
HDL	-0.007	0.962
Iron (ug/dl)	0.001	0.995
TIBC (ug/dl)	-0.128	0.376
ferritin (ng/ml)	0.324	0.022*
Transferrin saturation %	-0.01	0.946
Right CIMT	0.676	<0.001**
Left CIMT	0.669	<0.001**

*P value of < 0.05: statistically significant; **P value of < 0.001: statistically highly significant; rs =superman correlation coefficient; CRP; C - reactive protein, ESR; erythrocyte sedimentation rate, TG triglyceride, HDL; high density lipoprotein, LDL; low density lipoprotein BMI; body mass index, CIMT; Carotid intima-media thickness.

serum hepcidin levels and RA disease activity have also been published with controversial results.

Receiver operating characteristic curve analysis of the optimal cutoff of CIMT and Hepcidin levels. Hepcidin level ≥298.5 showed significant AUC that could discriminate between patients and control with sensitivity 72%, specificity 76%. van Santen et al., showed that hepcidin levels below 2.4 nmoles/liter discriminated IDA and IDA/ACD patients from ACD patients with RA.[19]

In the present study serum hepcidin levels showed significant positive correlation with (BMI, disease duration, cholesterol). No significant correlation was found between hepcidin and age, LDL, HDL (ug/dl). Rodríguez-Mortera et al., reported that hepcidin level was correlated with lipid metabolic parameters in their patients. The associations between hepcidin and [TG, TG/HDL ratio, VLDL-C and LDL2 (smaller sized LDL)] may indicate a link between atherogenic dyslipoproteinemia and hepcidin levels.[20]

In the present study hepcidin level showed significant positive correlation with ferritin. However, no significant correlation was found between hepcidin and (Iron, TIBC and Transferrin saturation). In agreement with our results, Galushko et al., reported that, in RA patients with anemia there was a strong positive correlation between serum hepcidin and ferritin levels. However, they added a strong negative correlation between serum hepcidin and Hemoglobin levels.[21] Contrarily, previous studies demonstrated associations between HEP and anemia markers or iron metabolism.[22, 23]

The present study showed that hepcidin level showed significant positive correlation with right CIMT and left CIMT. In line with our findings, a cross control prospective study showed that CIMT was correlated with high Serum Hepcidin-25(SH-25): a bioactive isoform of hepcidin. In this study, SH-25 was increased in hemodialysis patients who died of CVD. Furthermore, CIMT and SH-25 were independently correlated in this patient group. Finally, chronic hemodialysis patients with a median follow-up of 3 years, SH-25 levels were associated with the incidence of CV events, even after stepwise adjustments of clinical and anemia-related parameters, including inflammation.[24]

The present study showed that hepcidin level showed significant positive correlation with CRP. No significant correlation was found between hepcidin and ESR. In line with our findings, Erdogan et al., reported that BMI and CRP level were found to be statistically significantly higher in the high hepcidin group. However, they opposed our results as they showed that age was significantly higher in high hepcidin level.[25] These results are somehow inconsistent with some of the previously reported studies, which suggested that serum hepcidin may act as a surrogate marker for RA activity.[15, 27, 28]

The present study showed that CIMT ≥0.55 showed significant AUC that could discriminate between patients and control with sensitivity 82%, specificity 48%. Previous study by Mohan et al., demonstrated that at a cut-off value of CIMT greater than or equal to 0.57, the sensitivity and specificity were 84.4 [95% confidence intervals (CI), 67.2 - 94.7] and 90.6 (95% CI, 75.0 - 98.0), respectively. The 75th percentile value of CIMT in normal control subjects was found to be 0.55 mm.[11] Also, van Sijl et al., concluded that the mean CIMT in RA patients was 0.71 mm (95% CI: 0.65-0.77 mm) and in control subjects 0.62 mm (95%CI: 0.56-0.68 mm), resulting in an overall CIMT difference of 0.09 mm (95%CI: 0.07-0.11 mm.[28]

The present study revealed that univariate analysis was conducted for prediction of factors associated with increased CIMT in rheumatoid patients. BMI, disease duration, CRP, cholesterol level, ferritin and hepcidin level were significant predictors. However, in multivariate analysis CRP, cholesterol level, ferritin and hepcidin level were significant predictors. In line with our findings, Previous study showed that ferritin level ≥200 mg/L reported being associated with 2.2 times rise in the probability of acute myo-

cardial infarction even when adjusted for additional risk factors.[29] Gonzalez-Gay et al. showed that long-standing RA patients with mean CRP levels greater than 15 mg/dl had higher CIMT values than those with lower CRP level.[30] Arora et al showed that atherogenic indices such as LDL/HDL ratio, atherogenic index, and CIMT were significantly higher in the RA patients.[31]

Given the heightened cardiovascular risk observed in rheumatoid arthritis patients, clinicians should routinely assess traditional cardiovascular risk factors such as lipid profiles, blood pressure, and BMI. Comprehensive cardiovascular risk assessment and management should be integrated into the overall care plan for RA patients.

Further research is warranted to explore the potential role of hepcidin as a biomarker in RA. Investigating its association with disease progression, inflammation, and cardiovascular risk may provide valuable insights into RA management and risk stratification. Encouraging lifestyle modifications, including smoking cessation, weight management, and physical activity, should be a priority in RA patient care. Early intervention and aggressive management of RA, along with vigilant monitoring of cardiovascular risk factors, can help mitigate the increased cardiovascular burden associated with the disease.

One of the primary limitations of this study is the relatively small sample size. A larger and more diverse cohort could provide a more comprehensive understanding of the associations observed and enhance the generalizability of the findings. Longitudinal studies are needed to assess changes over time and determine the impact of interventions on cardiovascular risk in rheumatoid arthritis patients.

Conclusion

Higher hepcidin level was correlated with CIMT in patients with RA. Patient Age, duration of the RA disease, cholesterol level and hepcidin were linked to an increase in CIMT in rheumatoid patients in Multivariate logistic regression analysis; therefore, it may be an atherosclerotic risk marker in those patients. Further longitudinal follow with these patients over some time to look for clinical events that reflect the consequences of atherosclerosis is recommended.

Disclosures

Ethics Committee Approval: This study was approved by Menoufia Faculty of Medicine's Ethics Committee (Date: August 2021 - Number:10/18 INTM).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – S.S., E.E., E.M.E.Z.; Design – E.E., E.Z., E.M.; Supervision – S.S., F.E., E.E., E.Z., E.B.; Materials – E.M., E.B., F.E.; Data collection &/or processing – E.M., E.B., F.E.; Analysis and/or interpretation – E.M., S.S., S.M., E.E.; Literature search – E.M., S.S., F.E., E.E.; Writing – E.M., E.E., F.E., S.M.; Critical review – E.M., E.E., F.E., S.M., S.S.

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