Original Research Paper

Volume - 12 | Issue - 12 | December - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Medicine

SERUM HEPCIDIN AS A MARKER OF INFLAMMATION IN SEROPOSITIVE RHEUMATOID ARTHRITIS- THE NEW KID ON THE BLOCK?

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ABSTRACT **Introduction:** Assessment of disease activity in Rheumatoid Arthritis (RA) is required for the 'treat to target' strategy and includes clinical assessment as well as laboratory markers. These markers have fallacies, and thus confound disease activity scores. The search for an objective marker of inflammation continues. **Aims And Objectives:** This study is designed to evaluate if serum hepcidin can fulfill the role of a marker of inflammation in seropositive RA. **Materials And Methods:** Eighty two cases of seropositive RA fulfilling the inclusion and exclusion criteria for the study, and twenty five apparently healthy controls were taken into the study. Disease activity was assessed clinically and Clinical Disease Activity Index (CDAI) was calculated. Cases were also divided into 4 groups based on CDAI. C - Reactive protein (CRP), Erythrocyte Sedimentation Rate (ESR), Serum Ferritin, and Serum Hepcidin levels were measured. Data was tabulated using MS-Excel worksheet. Appropriate statistical tests were used to compare the parameters among cases and controls and across various disease activity groups. **Results:** The cases and controls were matched to age. Serum Hepcidin was significantly higher in the cases than in the controls. They also differed significantly between various disease activity groups. They correlated positively and significantly with CRP and ESR. Serum Ferritin levels did not vary significantly between cases and controls. **Conclusion:** Serum Hepcidin has the potential to be a marker of inflammation, though more studies including prospective ones will be required. Greater acceptance and availability will bring down the cost of testing.

KEYWORDS : Rheumatoid Arthritis, Serum Hepcidin, Serum Ferritin, Clinical Disease Activity Index.

INTRODUCTION

Assessment of disease activity in RA is required for the treat to target strategy. A number of disease activity scores are used which include clinical assessment in form of tender and swollen joint counts as well as laboratory markers like ESR and CRP. Prominent among these are DAS28 and CDAI, both of which are validated scores[1]. The former uses laboratory markers of inflammation while the later does not. However, acute phase reactants like ESR and CRP may be discordant in active disease[2], and thus contribute to fallacy in disease activity scores based on them. The search for an objective marker of inflammation continues, and includes serum hepcidin levels[3,4] whose synthesis is thought to be induced by IL-6[5]. Our study is designed to evaluate if serum hepcidin levels can act as an inflammatory marker in patients of Rheumatoid Arthritis (RA) in the Indian population.

Aims And Objectives

To evaluate if serum hepcidin levels can be used as a marker of inflammation in patients of seropositive RA without anaemia.

Material And Methods

A cross-sectional observational study was conducted in the Rheumatology Clinic of ABVIMS, Dr RML Hospital, a tertiary referral hospital in New Delhi, India. The subjects included patients of RA visiting the clinic. 82 cases and 25 controls were included in the study. Inclusion criteria included all patients fulfilling the American College of Rheumatology (ACR) 2010 criteria, positive for Rheumatoid Factor (RF) and/or anti citrullinated peptide antibodies (ACPA) and above 18 years of age, and of either gender. Exclusion criteria included patients having anaemia (Haemoglobin less than 11 gm/dL), renal abnormalities (serum creatinine>1.5 mg/dl), hepatic abnormalities, (SGOT, SGPT 3 times the upper limit of normal), hypothyroidism (TSH>6uIU/ml), lymphoproliferative or malignant disease, patient with any concurrent infection, patients who received blood transfusion, iron supplementation, or erythropoietin in last 3 months, patients with history of recent blood loss, and patients of RA having overlap with other connective tissue disorders. The patients were assessed by single observer to avoid inter observer variability and Clinical Disease Activity Index calculated. CRP was measured by ELISA, and ESR by Westergren's method. Samples were stored at -20˚C till measurement of hepcidin levels. Serum hepcidin was measured by DRG Hepcidin 25 (bioactive) HS ELISA kit, manufactured by DRG International Inc.

Data was tabulated using MS-Excel worksheet. Categorical variables were presented in number and percentage (%) and continuous variables were presented as Mean ± Standard deviation and Median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected, then non parametric test was used. Association of quantitative variables was teste using ANOVA/Kruskal Wallis Test (when the data sets were not normally distributed) between the three groups. Spearman rank correlation coefficient was used to assess the correlation of various quantitative parameters. P-value of <0.05 was considered statistically significant. Statistical Package for Social Sciences (SPSS) version 21.0 was used for statistical calculations. Log transformation was used to convert parameters with skewed distribution to normal distribution.

CDAI scores used in our study are as follows.
0.0-2.8= Remission 2.9-10= Low disease activity
10.1-22= moderate disease activity 22.1-76= High disease activity

At presentation, eligible patients of RA were divided into 4 groups based on CDAI scores. We compared the values of Hepcidin, Ferritin, ESR, and CRP, between the cases and controls, and also between each of the groups to find out whether there was any significant difference between them.

RESULTS

82 cases and 25 controls were included in the study after applying the inclusion criteria and exclusion criteria. There was no significant difference between the age of the controls and cases. However, there were 8 male cases, but no male controls. Thus the cases and controls were matched to age, but not gender. Out of the four parameters studied, only ESR was normally distributed. Hence Hepcidin, Ferritin and CRP were log transformed to yield a normal distribution and then compared. The values of the parameters are given in Table 1.

Table 1: Table showing comparison of various parameters between cases of seropositive RA and healthy controls.

Parameter	Controls	Cases	P value
Age	39.44 ± 10.66	42.38 ± 10.34	0.572
Hepcidin-Log	0.4537 ± 0.4125	0.7304 ± 0.4228	0.0045
Ferritin-Log	1.3448 ± 0.3683	1.3876 ± 0.3332	0.614
CRP-Log	0.3986 ± 0.2771	0.9755 ± 0.4815	<0.001
ESR	13.08 ± 5.7585	60.561 ± 26.4543	<0.001

Serum hepcidin levels were then compared across the various disease activity groups defined by CDAI. There were 6 patients in remission,

30 with low disease activity, 17 with moderate disease activity, and 29 with high disease activity. The value of Log Hepcidin level in the group of patients in remission was 0.7355 ± 0.5149, in the low activity group was 0.7691 ± 0.4485, in the moderate activity group was 0.9029 ± 0.3597, and in the high disease activity group was 1.2813 ± 0.4278 (Figure 1) which was statistically significant (P < 0.001).

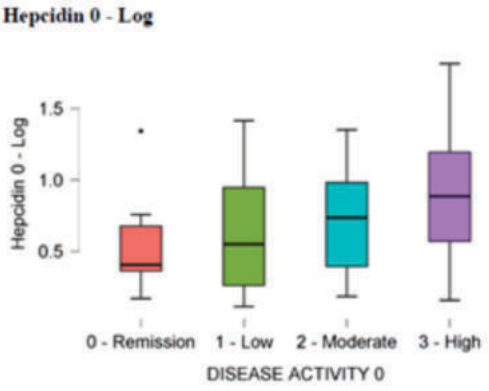

Figure 1: Box plot showing serum hepcidin levels in various disease activity groups in patients of seropositive RA.

Correlation study between the log values of Hepcidin revealed significant correlation between Serum Hepcidin (log) and already established markers of inflammation ESR, and CRP [Table 2].

Table 2: Correlation of log hepcidin values with inflammatory parameters

Parameter	R-value	P value
CRP	0.343	<0.001
ESR	0.280	0.004

DISCUSSION

There is always a continuing search for an objective parameter to indicate disease activity in RA, and Serum Hepcidin is one such candidate molecule which has been studied. While some studies have shown significant correlation of Hepcidin levels with disease activity in patients of RA[6], and significant differences between patients of RA and healthy controls[7], and between RA and undifferentiated arthritis[8], with the results holding true irrespective of presence of anaemia[9], some studies have shown the opposite results[10].

To be accepted as a marker of inflammation, Hepcidin should differ significantly between cases and controls, and should correlate with Clinical Disease Activity, as well as with other proven inflammatory markers of ESR and CRP.

In our study, Serum Hepcidin levels varied significantly between cases and controls. Moreover, they varied significantly between clinical disease activity states. Hepcidin levels also correlated with CDAI when analysed as a whole, and showed a positive correlation with ESR, and CRP, which are established inflammatory markers in use.

To obviate the confounding effects of anaemia, only those patients who had Haemoglobin levels of 11 gm/dL or more were included in the study. Ferritin in our cases did not differ significantly between cases and controls.

The patients in our study were positive for RF and/or ACPA, and there were no seronegative RA cases. The cases and controls were matched to age, but not gender. These, along with more cases than controls constitute the limitations of our study. However, it may be noted that this study was conducted during the covid19 pandemic and hence the sample sizes were small. Calling healthy controls to our hospital which was functioning as covid19 treatment centre would have been unethical.

We conclude that Serum Hepcidin levels has the potential to be a marker of inflammation in patients of seropositive RA, though more studies including prospective studies with larger sample size needs to be undertaken. Wider acceptance of serum hepcidin level testing will lead to wider availability and by economics of scale will bring down the cost of testing from the present INR (₹) 700.

Acknowledgement: The authors acknowledge the help of Dr Eashan Srivastava, Central Health Service, Ministry of Health & Family Welfare, Government of India, for the statistical analysis done in this article.

Disclosures: None
Conflict of interest: None

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