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# Correlation of Serum Hepcidin Levels with Inflammation and Iron Status in Patients with Esrd


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**ABSTRACT**

**Background and objectives:** Hepcidin is a small, cysteine-rich cationic peptide synthesized in the hepatocyte, is upregulated in uremia, and its presence provides an explanation for why chronic kidney disease patients absorb iron poorly and develop functional iron deficiency. Recently, measurements of hepcidin in blood have been developed, and it could be more informative of iron status in CKD. The present study was done to assess serum hepcidin levels in ESRD patients and its correlation with iron status and inflammation

**Methods:** The study included 72 individuals, 26 newly detected ESRD not on parenteral iron, 26 ESRD patients who already received prior parenteral iron or blood products, 20 healthy control subjects from September 2009 to May 2012. Renal function tests, serum iron, serum ferritin, serum transferrin along with prohepcidin levels were measured.

**Results :** Hepcidin levels have been found to be elevated in ESRD patients. High hepcidin levels suggest presence of functional iron deficiency. There was significant correlation between levels of hepcidin and iron status with inflammatory markers.


**KEYWORDS** End stage renal disease, Hepcidin, Ferritin, Iron, Anemia

## Introduction

Hepcidin is a small, cysteine-rich cationic peptide, identified in human blood using mass spectrometry (MS). It possesses anti-microbial properties, but is also found to be a regulator of iron utilization, binding to ferroportin, and modulating activity of other proteins involved in iron availability such as divalent metal transporter-1 and transferrin [1,2]. Its production is influenced by a variety of stimuli, including anemia, hypoxia, and inflammation. It is upregulated in uremia, and other chronic inflammatory states [3], and this could explain the poor oral iron absorption in CKD patients.

Tomosugi *et al.* found serum hepcidin levels to be elevated in hemodialysis patients. [4] This has been confirmed by various other groups, although the absolute values of hepcidin varied up to 10-fold depending on the assay used. The values for hemodialysis patients are in the range of 27 - 158 ng/ml, nondialysis CKD patients have values between normal and hemodialysis[5]. Some papers report changes in hepcidin levels after erythropoietin or intravenous iron, while there are conflicting data regarding removal of hepcidin by dialysis. Kato *et al.* [6] and Ashby *et al.* [7] reported no effect, whereas Weiss *et al,* [8] and Peters *et al* [9] reported a reduction in hepcidin levels after dialysis. The present study was done to assess serum hepcidin levels in ESRD patients and its correlation with iron status and inflammation.

## Material and methods

This study was a cross sectional study of 52 End Stage Renal Disease Patients and 20 healthy controls, conducted at Sri Ramachandra Medical College in the Department of Nephrology from September 2009 to May 2012. All patients and healthy controls who consented for the study were screened and investigations done. Patients with evidence of active infection and trauma, history of parenteral iron injection during last two weeks, history of blood transfusion in the last one month, history of malignancy, recent overt blood loss, hemoglobinopathies and history of transplant were excluded from the study.

All patients underwent a thorough physical examination. Values of Serum hemoglobin, Serum Iron, Serum TIBC, Serum Ferritin, Serum CRP, Transferrin saturation, Serum Albumin, Serum Pro Hepcidin, MAMC (mid-arm muscle circumference) and Body mass index were recorded. Ferritin is estimated by an immunometric enzyme immunoassay, Serum CRP was estimated using quantitative CPR assay kit, Pro-hepcidin was estimated using the DRG Hepcidin Pro-hormone enzyme immune assay kit. For individuals on dialysis receiving oral iron, it was withheld for a week before sampling Hemoglobin, serum iron, total iron binding capacity (TIBC), percentage transferring saturation (TSAT) and serum ferritin, serum CRP levels. The collected data was analyzed using Statistical Package of Social Sciences (SPSS) 17.0 for windows.

## Results

The mean age of patients in the study group was 50.55 ± 14.27 years (25 – 78 years). The mean serum hemoglobin was 10.2 g/dl ± 2.2g/dl (5.3 – 13.8 g/dl), with a mean serum iron of 88.1 ± 29.6 mcg/dl (30 – 142 mcg/dl). The mean transferrin saturation was 31.1 ± 9.8% (3.01 - 62%). The mean Prohepcidin level was 319.8 ± 61.06 ng/ml (220.7 - 523.0 ng/ml) (Table 1). In individuals who did not receive parenteral iron therapy the mean serum Prohepcidin was 338.5 ± 10.7 ng/ml and in those who received iron therapy, it was 364 ± 51.1 ng/ml (Table 2).

The serum hepcidin levels obtained were correlated with various parameters such as age, MAMC, BMI, S. Albumin, Hemoglobin, S. Iron, Transferrin saturation, TIBC, Serum Ferritin and Serum CRP. Serum hepcidin had a significant positive correlation with serum CRP (Figure 1) and serum Ferritin (p<0.001) (Figure 2) (Table 3).

**Discussion**

Hepcidin has a critical role in the pathogenesis of anemia of chronic disease. Indian subjects have higher microinflammation and body fat percentage as compared to Caucasians.[10] In our study, increased CRP levels were found in both study groups when compared with control. The cause of elevated mean CRP could be exposure to dialysis membranes, activation of immune cells, and possible co-existing subclinical infection.

A similar study done by V. Jha et al revealed significantly increased inflammatory activity with higher CRP in 74 ESRD patients (p value of < 0.001).[11] Similar findings is noted in our study with significant correlation with hepcidin and serum CRP levels (p< 0.001).

In our study, ESRD subjects in both groups had adequate iron stores with TSAT > 20%, with a TSAT > 35.6% in subjects who had received parenteral iron therapy. A study done by John GT et al revealed that 75% patients showed evidence of iron overload during pre-transplant evaluation.[12]

In this study, the major finding was elevated prohepcidin, a precursor of hepcidin. Subjects who did receive parenteral iron showed a higher hepcidin levels (364.0 ng/ml) compared with subjects who did not receive parenteral iron (338.5 ng/ml) and control revealed a mean value of 237.8 (ng/ml) (p < 0.01).

Malyszko J et al, showed elevated hepcidin in CKD patients, which is now considered to be a link between inflammation and anemia in CKD patients. [13] Weiss et al found that Hepcidin levels in chronic HD patients at baseline did not correlate with pro-hepcidin, but had significant association with serum ferritin level (p=0.04) and TSAT (p<0.001). [14] R. Damien et al, showed that higher hepcidin do not predict increase erythropoietin requirements.[15]

In our study, mean values of serum hepcidin was increased in CKD patients who were on hemodialysis and received i.v. iron therapy, which was significant. In our study S. ferritin was significantly elevated in subjects who received parenteral iron compared to individuals who didn't receive parenteral iron (p< 0.001).

Limitations of the study are that we included patient population consisting of newly detected ESRD and individuals already on hemodialysis, exact dose or formulation of I.V. iron is not known. Also, we measured hepcidin pro hormone, and didn't measure other inflammatory markers like interleukin-6.

**Conclusion**

The study emphasizes that serum hepcidin levels are elevated in ESRD patients. High hepcidin levels would reveal functional iron deficiency. There was significant correlation between levels of hepcidin and iron status with inflammatory markers.

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Age (years) | 72 | 25.00 | 78.00 | 50.5556 | 14.27288 |
| MAC | 72 | .00 | 30.00 | 18.2917 | 11.67209 |
| TSF | 72 | .00 | 8.00 | 4.0833 | 2.79209 |
| MAMC | 72 | .00 | 14.40 | 5.5375 | 4.61096 |
| BMI | 72 | 18.70 | 36.70 | 23.3236 | 3.76487 |
| Sr Albumin (g/dl) | 72 | 2.10 | 4.50 | 3.6458 | .58597 |
| Hemoglobin(g/dl) | 72 | 5.30 | 13.80 | 10.2944 | 2.29186 |
| Sr Iron (mg/dl) | 72 | 30.00 | 142.00 | 88.1847 | 29.06175 |
| TIBC | 72 | 30.90 | 370.00 | 287.2083 | 73.06656 |
| TSAT | 72 | 3.00 | 62.00 | 31.1250 | 9.86520 |
| Serum Ferritin (ng/ml) | 72 | 24.20 | 449.70 | 259.4125 | 153.49468 |
| Serum CRP (mg/l) | 72 | .80 | 50.60 | 22.3722 | 16.11977 |
| Serum Hepcidin (mg/ml) | 72 | 220.70 | 523.00 | 319.8014 | 61.06178 |
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**Table 1: Characteristics of the study participants..**

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| CKD pts without Iron | 26 | 338.5769 | 10.74703 | 2.10767 | 334.2361 | 342.9177 | 320.40 | 358.30 |
| CKD pts with Iron | 26 | 364.0346 | 51.17083 | 10.03543 | 343.3663 | 384.7030 | 321.40 | 523.00 |
| Controls | 20 | 237.8900 | 10.64132 | 2.37947 | 232.9097 | 242.8703 | 220.70 | 254.30 |
| Total | 72 | 319.8014 | 61.06178 | 7.19620 | 305.4526 | 334.1502 | 220.70 | 523.00 |

**Table 2: Serum Hepcidin level in patients with and without parenteral Iron supplementation**

| | | Serum Ferritin | Serum CRP | Serum Hepcidin |
|---|---|---|---|---|
| Serum Ferritin | Pearson Correlation | 1 | .875** | .840** |
| | Sig. (2-tailed) | | .000 | .000 |
| | N | 72 | 72 | 72 |
| Serum CRP | Pearson Correlation | .875** | 1 | .656** |
| | Sig. (2-tailed) | .000 | | .000 |
| | N | 72 | 72 | 72 |
| Serum Hepcidin | Pearson Correlation | .840** | .656** | 1 |
| | Sig. (2-tailed) | .000 | .000 | |
| | N | 72 | 72 | 72 |

**Table 3 - Pearson Correlation between Serum Hepcidin, CRP and Serum Ferritin**

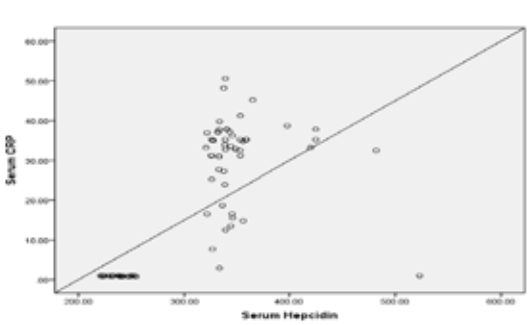


**Figure 1 - Pearson correlation between serum hepcidin and serum CRP showing significant correlation between Serum Hepcidin and Serum CRP**

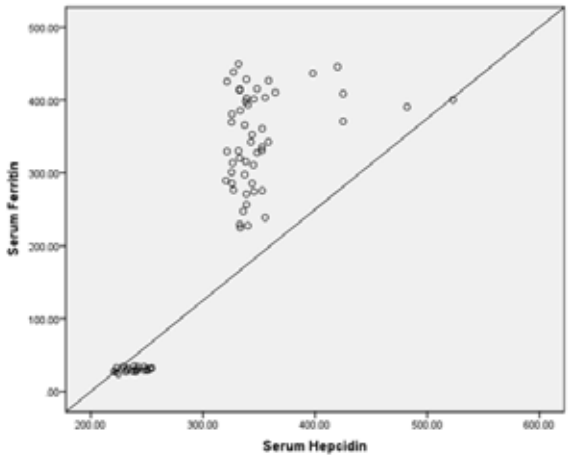


**Figure 2 - Pearson correlation between serum hepcidin and serum Ferritin showing significant correlation between Serum Hepcidin and Serum Ferritin**

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