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Original Article

Serum Hepcidin Levels in Patients with End-Stage Renal Disease on Hemodialysis

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ABSTRACT. Patients on hemodialysis (HD) are usually anemic because of defective erythropoeisis. Hepcidin is a polypeptide that regulates iron homeostasis and could serve as an indicator of functional iron deficiency in patients with end-stage renal disease (ESRD); this may also aid in the assessment of patient's response to erythropoietin (EPO). The present study was directed to investigate serum levels of hepcidin, iron status and inflammation markers such as C-reactive protein (CRP) in patients with ESRD on maintenance HD and to observe the correlation of serum hepcidin with conventional iron and inflammatory markers. A total of 42 patients of both sexes on maintenance HD and EPO therapy were enrolled; 42 age- and sex-matched healthy subjects were included as controls. Laboratory tests including complete blood count, serum hepcidin, total iron binding capacity (TIBC), serum ferritin, serum iron and CRP were performed. Serum hepcidin levels were significantly higher in patients with ESRD than in the control group (18.2 ± 2.8 ng/mL and 8.5 ± 2.3 ng/mL, respectively $P = 0.000$). The hemoglobin, hematocrit, serum iron, TIBC and transferrin saturation levels in the patient group were significantly lower than in the control group. Higher hepcidin levels were found in EPO non-responders (19.6 ± 2.4 ng/mL) while lower levels (16.9 ± 2.5 ng/mL) were seen in responders ($P = 0.001$). A positive and significant correlation was observed between the values of serum hepcidin and CRP. Our study indicates that higher hepcidin levels are found in ESRD patients on HD and in those not responding to EPO. Our findings suggest that hepcidin might play a role in the pathophysiology of anemia associated with chronic diseases as well as EPO resistance.

Introduction

Anemia is commonly seen in all stages of renal disease but is much more pronounced in patients with end-stage renal disease (ESRD).[1] Patients with anemia due to chronic kidney disease (CKD) are at increased risk of hospitalization, increased length of hospital stay, reduced quality of life and higher mortality.[2] The main causes of anemia in patients with CKD are decreased erythropoietin (EPO) production, chronic inflammation, shortened half-life of erythrocytes and iron deficiency.[3]

Hepcidin is a peptide secreted by the liver

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that regulates plasma iron.[4] Hepcidin production decreases in the presence of iron deficiency, hypoxia and ineffective erythropoiesis,[5] whereas increased production is stimulated by increased plasma and stored iron.[6] Increased levels of hepcidin results in iron retention in macrophages and decreased absorption of iron.[7] Hepcidin production is also increased by inflammation and high hepcidin concentrations limit iron availability for erythropoiesis, thereby playing a major role in the anemia of inflammation and EPO resistance.[8]

Hemodialysis (HD) is considered an inflammatory state and increased serum hepcidin levels have been found in patients with ESRD on maintenance HD.[9] These elevated levels in hemodialyzed patients could be due to functional iron deficiency anemia and low-grade inflammation.[10] Reticulo–endothelial blockade is seen during inflammation, which is mediated by hepcidin up-regulation, and results in inhibition of release of iron to transferrin.[8,11] Hepcidin also contributes to EPO resistance by regulating iron-restricted erythropoiesis and by its inhibitory effect on erythroid progenitor proliferation and survival.[8,11]

Uremia is a state of heightened inflammatory activation. This might have an impact on several parameters including those used in the management of anemia. Ferritin, for example, is a marker of body iron stores, but it also increases in acute inflammation and therefore becomes less valuable as an indicator of iron status during inflammation.[12] Serum iron and transferrin saturation are also influenced by inflammation. Inflammation also increases the C-reactive protein (CRP) and hepcidin levels,[9] but in spite of this complexity the existing data indicate that hepcidin has an advantage over ferritin in guiding treatment of anemia in patients with CKD as it directly reflects iron availability and the status of iron homeostasis, better than other conventional parameters.[13]

The current study was planned to determine the values of these conventional markers of body iron stores, degree of inflammatory activation and serum hepcidin in patients with ESRD on maintenance HD and to compare them with normal controls. This study was aimed at determining the usefulness of serum hepcidin levels in patients with ESRD and anemia (anemia of chronic disease) and to assess the possible correlation between hepcidin and markers of iron status and inflammation in patients with ESRD on HD. This study also compared the serum hepcidin levels in responders and non-responders to EPO.

Materials and Methods

Before the start of the study, permission was obtained from the Ethical Review Committee, University of Health Sciences, Pakistan according to the Helsinki amendments (2009).

It was a case control, comparative study and was conducted at the Department of Hematology, University of Health Sciences, in collaboration with the Sheikh Zayad Hospital, Lahore. It included 84 subjects, both male and female, between the ages of 18 and 60 years. These participants were divided into two groups. Group-I included 42 patients (22 males and 20 females) with ESRD on maintenance HD and on EPO injections for the treatment of anemia. This group was further divided into two sub-groups A and B depending on their response to EPO.

Sub-group A included EPO responders, and included patients who showed an increment of hemoglobin (Hb) more than 2 g/dL in a month after EPO therapy. Sub-group B comprised of EPO non-responders and included patients who did not show a rise in their Hb of 2 g/dL in a month after EPO therapy.

Group-II comprised of 42 age- and sex-matched healthy controls.

Patients with interrupted therapy, history of chronic liver disease, melena and hematemesis were excluded from the study. Similarly, patients who had received red cell transfusion within the last four weeks of the study were also excluded.

A consecutive sampling technique was used. Five milliliters of venous blood was drawn under aseptic conditions. Three milliliters was transferred to gel vaccutainers for the measurement of serum hepcidin, iron, ferritin, total iron binding capacity (TIBC) and CRP levels. The

Table 1. Demographic data of subjects included in the study.

	Patients	Controls
Number of subjects	42	42
Male	22	22
Female	20	20
Age (years; mean ± SD)	45 ± 12.3	48 ± 23.2
Range of age (years)	21–60	18–60

remaining 2 mL was transferred to the EDTA vaccutainer for the determination of Hb and hematocrit. Complete blood counts (CBCs) were performed on all the samples using an automated Hematology Analyzer Sysmex XT-1800i. Serum iron and TIBC were measured spectro-photo-metrically at 560 nm by using a commercially available kit of Randox on Metrolab 1600. Serum ferritin was measured by using the commercially available kit of Ferritin reagent pack lot #1186 on Eciq Vitros Immunodiagnostic system. The CRP levels were measured quantitatively by immuno-turbidimetric test on an Olympus (Germany) analyzer. The hepcidin level was measured by a Biorad-680 Microplate reader, USA using a Human Hepcidin ELISA kit from Creative Diagnostics, USA.

Statistical Analysis

The data were entered and analyzed using PASW 18.0. Mean ± SD were calculated for quantitative variables (Hb, serum hepcidin, serum ferritin, serum iron and TIBC). Two independent sample T tests were applied to observe mean difference between the two groups (controls and ESRD patients). Pearson correlation was applied to observe correlations between hepcidin and conventional markers of iron status and inflammation. A *P*-value of ≤0.05 was considered to be statistically significant.

Results

Eighty-four subjects were recruited in our study. Fifty percent of them were patients with ESRD on HD for the last three months and the other 50% were healthy subjects included as the control group. 52.3% were male and 47.6% were female in both the patient and the control groups. The mean age in the first group was 45 ± 12.3 years, while it was 48 ± 13.2 years in the control group. Demographic details of the participants are summarized in Table 1.

Mean level of Hb, hematocrit and serum iron profile (serum iron, TIBC, serum ferritin) were evaluated for the clinical assessment of iron status and anemia. The results of all the hematological and biochemical parameters analyzed are shown in Table 2. In ESRD patients, the mean Hb was 9.9 ± 1.4 g/dL, while a significantly higher mean Hb value of 13.2 ± 1.0 g/dL was observed in the controls (*P* <0.001). The mean value of hematocrit in patients and

Table 2. Serum hepcidin and conventional markers of iron status and inflammation in both groups.

Parameters	Patients	Controls	*P*-value
Hb (g/dL)	9.9 ± 1.4	13.2 ± 1.0	<0.001
Hct %	30.9 ± 4.5	39.5 ± 3.1	<0.001
Serum iron	35.2 ± 11.9	82.7 ± 15.9	<0.001
TIBC	238 ± 94	308 ± 52	<0.001
TSAT %	13.6 ± 6.1	22.9 ± 8.5	<0.001
Serum ferritin (ng/mL)	346 ± 305	85.6 ± 16	<0.001
CRP (mg/L)	13.3 ± 21.1	1.9 ± 2.1	<0.001
Hepcidin (ng/mL)	18.2 ± 2.8	8.1 ± 2.3	<0.001

This table shows mean values ± SD of the parameters in patients (42) and controls (42).
Hb: Hemoglobin, Hct: Hematocrit, TIBC: Total iron binding capacity, TSAT: Transferrin saturation, CRP: C-reactive protein.

Table 3. Correlation of serum hepcidin with conventional markers of iron status and inflammation.

Variable	r value	*P*-value
Serum ferritin	0.242	0.122
Serum TIBC	0.229	0.1566
Serum iron	0.043	0.788
Serum CRP	0.304	0.05

TIBC: Total iron binding capacity, CRP: C-reactive protein.

controls was 30.9 ± 4.5% and 39.5 ± 3.1%, respectively. A significant difference was observed between the two groups (P <0.001). Significantly increased levels of serum iron, TIBC and transferrin saturation (TSAT) were observed in the control group (P <0.001 each) as compared with the ESRD patients (Table 2). The levels of CRP (13.3 ± 21.1 mg/L) and serum ferritin (346 ± 305 ng/mL) were significantly higher in the ESRD group of patients in comparison with the controls (P <0.001).

In our study, the serum hepcidin level was 8.5 ± 2.3 ng/mL in the normal healthy control group and was significantly higher (18.2 ± 2.8 ng/mL; P <0.01) in the ESRD patients (Figure 1).

The levels of hepcidin showed insignificant correlation with serum iron level, ferritin level and TIBC (Table 3). However, the levels of serum hepcidin showed significant positive correlation with CRP (Figure 2), which is the conventional marker of inflammation (r = 0.304, P = 0.05) (Table 3).

The patients with ESRD (Group 1) who were receiving EPO therapy for at least three months were further divided into two subgroups (responders and non-responders) depending on their Hb response. Twenty patients (47.6%) who showed no rise in their Hb values were included in the non-responder group, while 22 patients (52.3%) presenting with an increase in Hb level were grouped as responders. Their mean hepcidin levels were 19.6 ± 2.4 ng/mL and 16.9 ± 2.5 ng/mL, respectively (Figure 3). A statistically significant difference was observed between the two groups (P <0.001; Table 4).

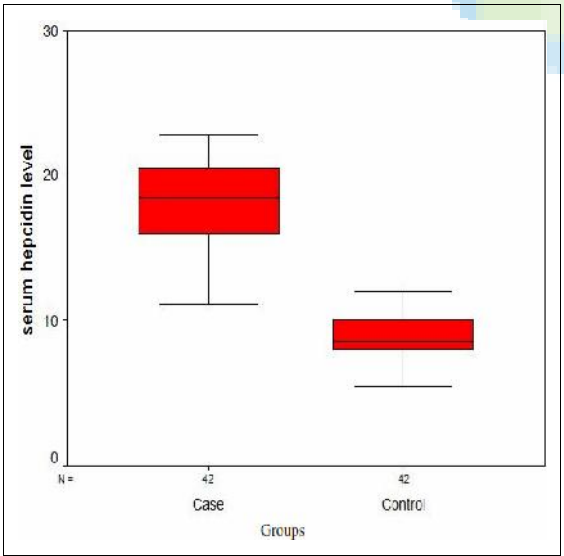

Figure 1. Box plots for hepcidin levels in patients and controls. Boxes show range of hepcidin (ng/mL) and horizontal lines inside the boxes indicate the mean value. Higher serum hepcidin levels are shown in the patient group (ESRD) as compared with the controls.

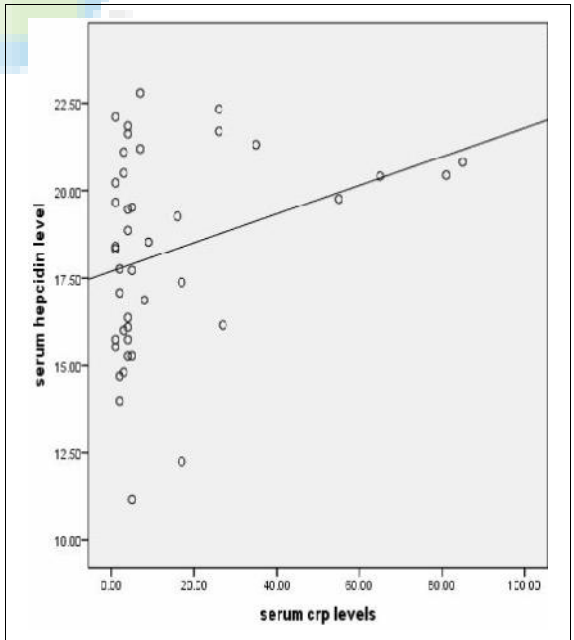

Figure 2. Scatter graph shows the relationship between serum hepcidin levels and serum CRP in patients with ESRD on hemodialysis.

Table 4. Serum hepcidin and conventional markers of iron status in responders and non-responders.

Variables	Responders	Non-responders	*P*-value
Hemoglobin (g/dL)	11.4 ± 0.9	8.8 ± 1.1	0.000**
Hematocrit %	33.7 ± 3.0	26.8 ± 3.6	0.000**
Hepcidin (ng/mL)	16.9 ± 2.5	19.6 ± 2.4	0.000**
Iron (µg/dL)	37.9 ± 12.5	33.0 ± 11.0	0.190*
TIBC (µg/dL)	278.9 ± 120	237.6 ± 93.0	0.224*
Ferritin (ng/mL)	332.6 ± 264	378.7 ± 346.7	0.634*

**Significant *P*-value, *Insignificant *P*-value, TIBC: Total iron binding capacity.

Discussion

Hepcidin levels are regulated by iron status and erythropoietic activity.[14] It is now well documented that hepcidin levels are reduced by anemia and hypoxia and increased by inflammation.[15] Renal anemia is considered a special form of anemia of inflammation.[16] The present study focused on the levels of serum hepcidin in patients with ESRD on maintenance HD for at least three months and their levels were then compared with controls.

In our study, we used the enzyme-linked immunosorbent assay (ELISA) method for the detection of serum hepcidin levels and found that the levels were significantly higher in patients with ESRD on HD as compared with healthy controls. Comparable results were also reported in other studies.[12] It has also been indicated that hepcidin levels were approximately two- to three-fold higher in patients with ESRD than in the controls.[13] Hepcidin levels are expected to be elevated in patients with ESRD due to limited hepcidin excretion in urine, tissue iron overload and inflammation.[17]

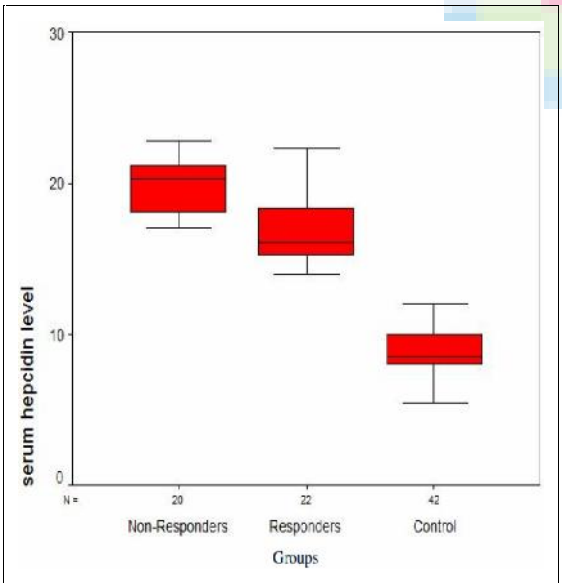

Figure 3. Box plots for hepcidin levels in non-responders, responders and controls. Boxes show range of hepcidin (ng/mL) and horizontal line inside the boxes indicates mean value. Higher serum hepcidin levels are shown in the non-responder group.

Among our group of patients, we found decreased levels of serum iron, TIBC and TSAT. However, serum ferritin levels were found to be elevated in this group. Findings consistent to ours have been seen in a study on patients with CKD.[18] The situation in which the TSAT is low and the serum ferritin is high is frequently seen among HD patients. High ferritin levels may be observed in this disease because of functional iron deficiency or reticulo–endothelial blockade. This commonly seen paradox of high serum ferritin and low TSAT has made it desirable to look for a substitute iron marker to predict better iron status of the patient.[19] Various other studies also support that current markers of iron metabolism like TSAT and ferritin do not predict iron status effectively[20] and that these conventional markers have certain limitations.[6] The diagnosis of iron deficiency using these markers is unproductive, as it can be affected by variables such as age, sex, inflammation and nutritional factors. In another study, it was concluded that determining hepcidin concentrations together with conventional markers associated with iron metabolism improved the identification of patients with iron deficiency by 26.1%.[21]

In this study, CRP was measured as the con-

ventional marker of inflammation and was found to be higher in patients than controls, and these levels were found to be significantly correlated with serum hepcidin. It is known that hepcidin synthesis is induced by inflammation, a process that is mediated by IL-6. As CKD is considered an inflammatory state, this positive correlation was expected.[8] Our results are comparable to other studies on patients with renal failure, which showed a correlation of hepcidin levels with CRP.[10] However, there are other studies in CKD patients where no correlation was observed between hepcidin and CRP levels.[8,17] This lack of correlation may be explained on the basis of differences in the half-lives of CRP and hepcidin.[17]

The use of recombinant human erythropoietin (rHuEPO) has brought a revolution in the treatment of renal anemia.[22] Increasing the dosage of erythropoiesis-stimulating agents (ESA) may seem to be the convenient path, because ESAs are considered safer than intravenous iron.[19] High doses of ESA are associated with increased risk of myocardial infarction, chronic heart failure, stroke and mortality.[23-25] Most of the patients with high serum ferritin and low TSAT levels demonstrate evidence of ESA resistance, and already are on very high dosages of an ESA with the Hb below the desired target. Identification of these ESA hypo-responsive/resistant patients is very important in the management of renal anemia. In our study, levels of serum hepcidin were found to be higher in non-responders than in responders with a statistically significant difference (P <0.001). A study conducted earlier concluded that hepcidin levels are thought to be a better predictor of EPO response.[11] In CKD, increased inflammation and possibly decreased clearance of hepcidin can lead to higher serum hepcidin levels, further contributing to iron-restricted erythropoiesis and EPO resistance.[8]

Conclusion

Serum hepcidin levels are increased in patients with ESRD on HD and, hence, may be used in the evaluation of anemia in such patients. Serum hepcidin provides useful information about the level and availability of iron during inflammation as compared with traditional markers of iron status. Levels of hepcidin are higher in non-responders to EPO than the responders and can be used as an important marker to assess the response to ESA.

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Conflict of interest: None

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