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ORIGINAL ARTICLE

Implication of serum hepcidin level in maintainance haemodialysis patients in association with different iron indices

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Abstract

Background: *Anaemia is a frequent complication in maintenance hemodialysis (MHD) patients and is associated with increased morbidity and mortality. Altered systemic iron metabolism and functional iron deficiency mainly related to subclinical inflammation makes it difficult to maintain proper control of anaemia. The discovery of hepcidin and it's functions has contributed to a better understanding of iron metabolism disorders in CKD anaemia.*

Objective: *To evaluate the association of serum hepcidin with different iron indices (serum iron, serum ferritin, TIBC and TSAT).*

Materials and Methods: *The study was carried out in the Institute of Kidney Diseases and Urology, Dhaka for a period of six months between July to December 2016. Serum hepcidin-25, current iron markers and other laboratory parameters were measured among study subjects. Absolute and functional iron deficiency patients were identified according to serum ferritin and TSAT. Statistical analysis was performed to find out whether serum hepcidin level significantly differ in MHD patients than healthy controls and also to evaluate it's correlation with other iron indices among MHD patients.*

Results: *Total 88 subjects (fifty MHD patients and thirty eight healthy controls) were enrolled into the study. Serum hepcidin level was significantly higher in MHD patients than healthy controls (19.3 (7.0 - 81.8) ng/ml vs 8.0 (2.4 - 33.6) ng/ml, P value <0.001). Hepcidin has significant positive correlation with ferritin in MHD patients (r=0.480; p=<0.001). Hepcidin was also positively correlated with serum ferritin both in absolute iron deficiency patients (r=0.786; p=0.036) and functional iron deficiency patients (r=0.764; p=0.006).*

Conclusion: *Serum hepcidin level is increased in MHD patients and associated with disturbance of iron metabolism. Hepcidin positively correlates with serum ferritin in MHD patients and may be used similarly as ferritin in guiding iron therapy.*

Key words: *Hepcidin, Ferritin, Absolute iron deficiency, Functional iron deficiency*

Introduction

Anemia is a frequent complication of chronic kidney disease and is associated with increased morbidity and mortality. Insufficient production of erythropoietin and iron deficiency is the main cause of anemia in maintenance hemodialysis (MHD) patients. Use of erythropoiesis-stimulating agents (ESA) has significantly improved the outcome of renal anemia. Unfortunately, a considerable percentage of MHD patients often show suboptimal response to ESA.[1]

Absolute and functional iron deficiency has been considered as the main cause of the hypo responsiveness to ESA.[2] Unfortunately, the two most commonly used markers of iron status, transferrin saturation (TSAT) and ferritin, often lack the needed specificity and sensitivity to predict the response to iron therapy in the CKD population. MHD patients are frequently assumed to have a functional iron deficiency whereby the supraphysiologic rate of red blood cell production driven by ESA therapy has outstripped the ability of transferrin to deliver sufficient iron for hemoglobin synthesis. In this case, iron supplementation may be beneficial; however, an extreme case of functional iron deficiency can occur when increased inflammation results in reticulo-endothelial blockade, a state in which iron release from stores is inhibited.

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Under such circumstances, iron supplementation would be ineffective and could lead to iron overload. Often the treatment of functional iron deficiency is empiric iron supplementation, because current iron parameters are unable to distinguish reticuloendothelial blockade from simple functional iron deficiency.[3,4]

To address the limitations in serum ferritin and TSAT and better target iron therapy, it is crucial to understand the molecular mechanisms behind iron balance, inflammation, and erythropoiesis in CKD. Hepcidin, an acute phase reactant protein produced in the liver, has emerged as a key regulator of iron homeostasis. Hepcidin's biologic actions are mediated by its binding to ferroportin, the principal cellular iron efflux channel. Once hepcidin is bound, it causes the rapid internalization and degradation of ferroportin.[6] Thus, by binding to ferroportin on the basolateral membrane of the duodenal enterocytes and on the cell membrane of the reticuloendothelial cells, hepcidin reduces iron absorption and iron release from macrophages, respectively. This leads to decreased iron bio-availability and iron restriction. Since, hepcidin levels are known to be elevated in CKD.[7] Hepcidin is now believed to play a central role in mediation of anaemia of CKD by reducing iron bio-availability and promoting iron restricted erythropoiesis. Increased inflammation and decreased clearance of hepcidin can lead to higher serum level of hepcidin in MHD patients.4,8 The objective of the present study was to determine the association of serum hepcidin with different iron indices in maintenance hemodialysis patients.

Materials and methods

It is a cross-sectional observational study carried out in National Institute of Kidney Diseases and Urology, Sher-E-Bangla Nagar, Dhaka from July 2016 to December 2016. CKD stage-5 patients on maintenance haemodialysis for more than 3 months, aged 18 years or above and age- sex matched healthy controls were included in this study. Patients who have history of acute blood loss or recent infection were excluded from the study. After approval of study by the ethical committee of the Institute, fifty hemodialytic patients and thirty eight healthy control fulfilling the inclusion and exclusion criteria were enrolled for the study. After proper explanation, maintaining confidentiality, measures to prevent harm to subjects, informed written consent was taken. Data was collected from each patient on the basis of history, findings of clinical examination and investigations including hematological and biochemical parameters.

From all the patients predialysis blood sample was drawn from the arterial needle prior to connecting the arterial blood tubing or flushing the needle and for healthy controls, blood sample was drawn from peripheral vein. Each blood sample was put into three different test tubes for haematological, biochemical investigations and allowed to clot for half an hour and then the samples were centrifuged for 15 minutes and serum was stored in ultra-deep freezer until analysis. Serum hepcidin level was measured by Enzyme linked immunosorbent assay (ELISA). After getting the result of iron profile, TSAT was calculated according to the formula. SPSS version 22.0 (SPSS Inc., IL, USA) was used for statistical analysis.

Results

Total of 88 adults (50 MHD patients and 38 healthy controls) were studied. The baseline characteristics of the subjects are shown in Table I.

Table I

Baseline characteristics of study subjects (n=88)

	Group-1 MHD patients (n=50)	Group-2 Healthy controls (n=38)	P value
Age (years)			
Mean ±SD	51.7±14.5	45.9±13.1	0.211
Gender			
Male	25 (50.0)	21 (55.3)	0.624
Female	25 (50.0)	17 (44.7)	
BMI (Mean±SD)	21.8±3.4	23.8±3.1	0.005

"Chi-square test and unpaired t test for level of significance

There was no difference in age and gender distribution among the study population. BMI was significantly (p<.05) lower in MHD patients compared to healthy controls. As shown in Table II , in healthy controls, serum ferritin level was 24.5±35.8 ng/ml, while a significantly higher serum ferritin value of 409.4± 342.9 ng/ml was observed in MHD patients (P<0.001). Serum hepcidin level of MHD patients was significantly higher than those in the healthy controls (19.3 (7.0 - 81.8) ng/ml vs 8.0 (2.4-33.6) ng/ml, P<0.001). There were no statistical differences between MHD patients and healthy controls with respect to TSAT and CRP level (P>0.05). Hb, iron, TIBC and serum albumin were significantly lower (p<.001) in MHD patients compared to control subjects (Table III). Serum hepcidin level was 11.4 (7.0-29.7) ng/ml in absolute iron

deficiency patients and 19.0 (9.3-75.6) ng/ml in functional iron deficiency patients.

Table II

Distribution of laboratory parameters among study subjects (n=88)

Laboratory parameters	Group-1 MHD patients (n=50) [mean+SD]	Group-2 Healthy controls (n=38) [mean+SD]	P Value
Hb (gm/dl)	9.0±1.3	14.2±1.4	<0.001
RBC (mill/cmm)	4.30±0.5	4.9±0.5	<0.001
HCT (%)	33.8±6.0	43.3±4.9	<0.001
MCV (fl)	79.0±9.1	86.8±99	<0.001
MCH (Pg)	36.9±3.4	35.3±4.2	0.059
MCHC (gm/dl)	32.0±2.3	32.5±2.4	0.336
Iron (ug/dl)	96.2±35.2	121.2±43.7	0.005*
Ferritin(ng/ml)	409.4±342.9	24.5±35.8	<0.001*
TIBC (ug/dl)	330.0±48.3	359.1±57.4	0.037*
TSAT (%)	30.0+12.9	34.7±13.3	0.056*
Albumin (gm/dl)	3.5±0.5	4.3±0.5	<0.001
CRP (mg/l)	23.4±25.6	10.3±11.5	0.060*
Hepcidin(ng/ml)	19.3 (7.0–81.8)	8.0 (2.4–33.6)	<0.001*

-Unpaired t test - for level of significance.

* Mann-Whitney U test - for level of significance

Spearman rank analysis indicated that serum hepcidin showed significant positive correlation with serum ferritin (r=+0.480; p=<0.001) in MHD patients.

Table III

Correlation of hepcidin level with different indices in absolute and functional iron deficiency patients

	Absolute iron deficiency (n=7)		Functional iron deficiency (n=11)	
	r value	p value	r value	p value
Ferittin	+0.786	0.036	+0.764	0.006
Iron	+0.875	0.014	+0.046	0.894
TIBC	-0.821	0.023	+0.369	0.264
TSAT	+0.357	0.432	-0.500	0.117
CRP	+0.593	0.161	+0.548	0.081

Spearman's ranks correlation for level of significance.

However, there were no significant correlation between serum hepcidin level and serum iron, TIBC, TSAT and CRP in MHD patients. Hepcidin had significant positive correlation with serum ferritin in absolute iron deficiency patients (r=+0.786; p=0.036) and also in functional iron deficiency patients (r=+0.764; p=0.006).

Discussion

In this current study, it was observed that Mean age of the MHD patients were 51.7 ± 14.5 years similarly, in the study of Yuxia et al. it was observed that MHD patients were older.9 Male and female were same in MHD patients. No gender predominance was found in the study of Ali et all.7 Mean BMI was significantly lower in MHD patients (21.8+3.4 kg/m2). Ali et al found the mean BMI 24.9 ± 5.8 kg/m² in HD group which is higher than the present study.10 In another study, Weerd et al found that the mean BMI was 25.0 ± 4.8 kg/m² in chronic haemodialysis(CHD) patients which is also higher than our MHD patients". This difference indicates most of our MHD patients suffer from calorie deficiency.

In the present study, we found mean haemoglobin was significantly low in MHD patients (9.0±1.3 gm/dl) and normal in healthy controls (14.2±1.4 gm/dl) which is different from the study done by Yuxia et al.9 They found mean haemoglobin was 12.4±1.4 gm/dl in MHD patients and 12.8±1.8 gm/dl in healthy controls. So, most of our MHD patients are anemic. They can not achieve the modest target of haemoglobin 11 to 12 gm/dl despite iron and ESA therapy. Haematocrite (Hct) and MCV were lower in MHD patients (33.8±6.0% and 79.0±9.1 fi respectively) than healthy controls (43.3±4.5% and 86.8±9.9 fl respectively). Among MHD patients, both Hct and MCV were found lower than the normal reference value in 18 cases. Probably they had microcytic hypochromic anaemia. Other RBC indices like MCH and MCHC were found with no statistically significant difference between MHD group and control group. Similar findings regarding haemoglobin and RBC indices were also observed by several studies.[12,13]

In the present study, we found serum hepcidin level was significantly higher in MHD group [19.33 (7.0-81.8) ng/ml) than healthy control group [8.0 (2.4-33.6) ng/ml). Yuxia et al. found that serum hepcidin was significantly higher in MHD patients than the control group (424±174.2 ng/ml vs 72.4±12.3 ng/ml; P<0.01).9 Manolov et al. observed statistically significant differences in serum hepcidin levels in CKD stage-V patients on chronic dialysis (282.5±81.1mg/l) and in control group (12.7±8.7mg/I).14 Ashby et al, reported that the median active form of hepcidin was 26.5 and 58.5 ng/ml in patients with CKD and under hemodialy

sis, respectively.[7] Ganz et al, Tomosugi et al, Rubab et al, and Weiss et al, also found significantly higher hepcidin level in MHD patients than healthy controls.[8,15-17] So, our findings are consistant with the above studies, but we find a difference in the absolute concentrations of hepcidin in different studies. The reason for these differences is not clear; however, it clearly complicates the direct comparison of hepcidin values between studies and the establishment of reference serum levels.[18]

Absolute iron deficiency (TSAT 20%, ferritin 200 ng/ml) was found among 7 MHD patients and functional iron deficiency (TSAT 20%, ferritin>200 ng/ml) was found among 11 MHD patients. Median hepcidin level was 11.4 (7.0 - 29.7) ng/ml in AID patients and 19.0 (9.3-75.6) ng/ml in FID patients. So, functional iron deficiency was associated with higher hepcidin level than absolute iron deficiency. In this current study we found serum hepcidin had strong positive correlation with serum ferritin in MHD patients ($r=0.480$; $p=<0.001$). Similar findings in chronic haemodialysis patients ($r=0.672$; $p<0.001$ and $r = 0.631$; $p = 0.004$) were observed by Weiss et al, and Ali et al respectively in their studies.[10,17]

In absolute iron deficiency (AID) patients, we observed hepcidin had significant positive correlation with serum ferritin ($r=0.786$: $p=0.036$) and serum iron ($r=0.875$: $p=0.014$), but strong negative correlation with TIBC ($r=-0.821$; $p=0.023$). Jairam et al also showed that serum hepcidin was significantly lower in absolute iron deficiency patients, which is similar to the current study. They mentioned that in absolute iron deficiency state erythropoeitic signal from erythroblast decrease production of hepcidin from hepatocyte to activate the ferroportin receptor that may aid in supply iron into serum.

In functional iron deficiency (FID) patients serum hepcidin had significant positive correlation with serum ferritin ($r=0.764$: $p=0.006$). Therefore, serum hepcidin provides useful information about the level and availability of iron during inflammation as compared with traditional markers of iron status like ferritin. We found hepcidin had weak positive correlation with serum iron ($r=-0.046$: $p=0.894$) and TIBC ($r=-0.369$: $p=0.264$), but weak negative correlation with TSAT ($r=-0.500$: $p=0.117$) in FID patients. In the current series mean C-reactive protein (CRP) was found higher in MHD group (23.4±25.6 mg/l) than control group (10.3±11.5 mg/l). Weerd et al observed high CRP in haemodialysis patients which had similarity with our study.[11] So evidence of marked inflammatory activation was demonstrated in MHD patients. We observed serum hepcidin level was positively correlated with CRP in MHD patients but statistically not significant. Correlations of hepcidin with inflammatory markers in CKD have also been found in other studies.[16]

There are several limitations in this study: First, the sample size of participants was relatively small, which may have a lower statistical power. Second, the present study was conducted at a short period of time. Third, the study cases were selected from one dialysis centre, so the study result may not represent the exact picture of the country.

Conclusion

Serum hepcidin positively correlates with serum ferritin and can be used similarly as ferritin in guiding iron therapy in MHD patients. Specifically, lowering serum hepcidin level help in improving gastrointestinal uptake of iron and it's release from reticuloendothelial cells, thus limiting the need for intravenous iron, overcoming functional iron deficiency and improving ESA resistance.

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