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Original Research Article

EMERGING ROLE OF HEPCIDIN IN ANEMIA OF CHRONIC KIDNEY DISEASE

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Abstract

Background: Anemia of chronic kidney disease is multifactorial in origin, and establishing an actual cause is a challenging task. Iron indices provide insight into differentiating iron deficiency and chronic disease Anemia in clinical practice. We used semiquantitative bone marrow iron estimation, iron indices, and serum hepcidin and C reactive protein to ascertain the cause of Anemia in CKD. **Method and Materials:** A prospective study was conducted on CKD stages 3-5 with Anemia after excluding non-renal causes of Anemia. A semiquantitative assessment of iron was done in bone marrow, and S. Iron indices, S. Hepcidin, and C-reactive protein were evaluated in all subjects. Patients were classified into three groups on the basis of bone marrow iron: 1. Normal Iron status, 2. Iron deficiency Anemia, 3. Anemia of Chronic Disease. These groups were then evaluated using different parameters of iron indices, Hepicidin, and CRP to assess the utility of Hepicidin in typing Anemia and the role of Hepicidin in Anemia. **Results:** Iron deficiency Anemia was the most common type, followed by Anemia of chronic disease and Anemia with normal bone marrow iron. Transferrin saturation is significantly low in the IDA group, with a mean value of 16.20% ($p < 0.05$) when compared to Anemia of chronic disease and normal iron status. Serum ferritin was significantly higher ($p<0.006$) in Anemia of chronic disease (301mg/dl) and the lowest in the iron deficiency group (108.56mg/dl). Serum Hepcidin was significantly higher ($p<0.05$), 154.8ng/dl in normal iron status when compared to Anemia of chronic disease 101.76ng/dl and iron deficiency anemia 78.71ng/dl. Ferritin and CRP correlate well with Anemia of chronic disease, while Transferrin saturation correlates with IDA. **Conclusion:** Interpretation of Iron Indices in CKD is only sometimes conclusive and may be uncertain occasionally. Iron deficiency is the most common cause of Anemia in our CKD patients. Hepcidin emerged as a potential marker to differentiate between functional Anemia in CKD due to inflammatory block or predominant due to Hepcidin only.

INTRODUCTION

Chronic Kidney disease (CKD) is a global public health problem, with published data indicating that 8-9% of patients with stage 1 CKD are anemic, and the prevalence of Anemia increases to 50%-92% in patients reaching stage 5 CKD.[1,2,3] Iron deficiency anemia is a common cause of Anemia in CKD; on the one hand, iron supplementation helps rectify Anemia, but on the other hand, it may prove hazardous by increasing tissue iron, leading to precipitation of infection and tissue injury mediated via free radicals.[4,5,6] Therefore, knowing the exact iron status in CKD patients is imperative for proper management.

Iron indices are a significant tool for diagnosing iron deficiency anemia in today's era. Serum ferritin of less than 100 ng/ml and TSAT of less than 20% indicate an iron deficiency in CKD6. However, these parameters become confounding in CKD due to the inflammatory state often existing in CKD.[4,5,6] One major molecule whose role is evolving in Anemia of chronic kidney disease is Hepcidin. Hepcidin is synthesized in the liver and is an acute-phase protein that is considered a key regulatory protein in iron homeostasis. Several mechanisms regulate hepcidin synthesis, which includes iron

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deficiency, hypoxia, and erythropoietic activity. A decrease in the blood hepcidin level results in the release of stored iron and an increase in dietary iron absorption. Infection and inflammation, on the other hand, cause an increase in hepcidin synthesis that leads to a deficiency of iron available for erythropoiesis and is considered to be the mechanism underlying the reticuloendothelial iron sequestration, impaired intestinal iron absorption, and low serum iron levels that are characteristic of the Anemia of chronic disease.[18,19,20] The role of serum hepcidin, its cutoff values, and the optimal assessment method are still evolving and might be the future of defining iron deficiency in CKD.

The last resort to evaluate actual iron status is tissue iron staining, and the absence of stainable iron in the bone marrow is still accepted as the gold standard for absolute iron deficiency. Few studies are available on serum iron indices with Hepcidin and some with tissue iron staining, but the data comparing all three modalities in the CKD population is minimal. In the current study, the actual iron status in CKD patients was evaluated prior to them being exposed to iron therapy by detecting the stainable iron in the bone marrow, and it was compared with serum iron indices, CRP, and Hepcidin levels.

MATERIALS AND METHODS

This Prospective cross-sectional study was conducted in the Department of Nephrology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, between 01 January 2016 and 31 December 2017. After taking due ethics committee clearance, adult chronic kidney disease patients between CKD stages 3-5 who did not require dialysis and had renal Anemia were enrolled after receiving written informed consent. All the participants were subjected to a detailed history, thorough physical examination, and investigation that included complete blood count with peripheral smear, absolute reticulocyte count, serum iron indices (iron, TIBC, Ferritin, and TSAT), serum B12 and folic acid, serum hepcidin, serum C-reactive protein (CRP), kidney function test, liver function test, plasma glucose, HbA1c, and ultrasonography whole abdomen.

In all participating subjects, the etiology of CKD was determined on the basis of clinical parameters and investigations, and its staging was done as per the estimated glomerular filtration rate (eGFR) calculated by using the Modification of Diet in Renal Disease (MDRD) equation. For statistical purposes, ten age and sex-matched controls were also selected. Anemia in CKD was defined as per KDIGO 2012 guidelines, with hemoglobin < 13gm /dl in Males and < 12 gm/dl in females. All patients were thoroughly investigated for etiology of Anemia, and subjects with non-renal causes were excluded. Patients with a history of blood transfusion in the past four months, patients receiving supplemental oral or parenteral iron therapy, erythropoietin stimulating agent, patients on renal replacement therapy, and patients with collagen vascular disease, malignancy, recent infection or antibiotic use were excluded from the study.

Quantitative measurement of Hepcidin was done by using MyBioSource human Hepc25. It is a solid-phase enzyme-linked immune-sorbent assay (ELISA) based on the principle of competitive binding. This assay employs the quantitative sandwich enzyme immunoassay technique. (Detection range: 4.69-300ng/ml). Semiquantitative iron estimation was done using bone marrow iron staining. Bone marrow was aspirated from the iliac crest; smears were prepared from marrow fragments and stained with Perls' Prussian blue stain. Siderotic granules in macrophages were graded on a scale of 0-6. Patients with a score of 0-1 were considered iron deficient; from 2-4, normal macrophage iron, and 5-6 were graded macrophage iron overload. Erythroblasts with green-blue particles on Perls' stain were defined as sideroblasts. According to the number of siderotic granules, sideroblasts were classified as type 0 (0 granules), type 1 (1-3 granules), or type 2 (>3 granules). The pattern of bone marrow iron distribution was classified as

1. Normal iron distribution or non-inflammatory functional Anemia: macrophage iron of 2-4, and sideroblasts in normal percentage.
2. Iron deficiency anemia -macrophage iron of 0 or 1, sideroblasts absent or present in a very low percentage.
3. Anemia of chronic inflammation macrophage iron ≥5, sideroblasts absent or present in very low percentage.
4. Iron overload macrophage iron ≥5, sideroblasts in normal or increased percentage.

The data were entered in an MS EXCEL spreadsheet, and analysis was done using IBM Statistical Package for Social Sciences (SPSS) version 22.0. The normality of variable distribution was tested using the Shapiro-Wilk W-test. Wherever possible, data were logarithmically transformed to achieve normal distribution. Data were reported as means ± SD; Analysis of variance (ANOVA) was used to compare differences between groups with $p < 0.05$ considered statistically significant, when appropriate.

RESULTS

Serum Ferritin and Transferrin are the general tools used to assess iron status in CKD patients; however, these tests are only sometimes helpful in the categorization of Anemia due to certain shortcomings of this parameter. We observed significant differences in the mean values of Ferritin in all 3 study groups, with the highest mean values in Anemia of chronic disease (301mg/dl) and the

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lowest in the iron deficiency group (108.56mg/dl). Serum ferritin was significantly raised in most patients of Anemia of chronic disease (>100ng/ml in 88%), as compared to other groups. This was significant when compared to the normal iron status group and the iron deficiency anemia group (p<0.006). Mean transferrin saturation (TSAT) was lowest in the IDA group at 16.20%, the highest value in Normal iron status at 29.27%, and the TSAT in the Anemia of chronic disease group was 26.76%, lying midway. Transferrin saturation is significantly low in the IDA group (p < 0.05) when compared to Anemia of chronic disease and normal iron status.

In our study, the mean hepcidin value was significant in Anemia of normal iron status when compared to Anemia of chronic disease and iron deficiency anemia (p<0.05). Mean serum hepcidin levels were 78.71±12.06 in the iron deficiency group, 101.76±26.15 in the Anemia of the chronic disease group, and 154.81±34.12 in the normal iron status. The hepcidin levels were higher in all three groups than in the control group. CRP values were highest in ACD at 1.4mg/dl, followed by 1mg/dl in Anemia of normal iron status and 0.4mg/dl in IDA. Mean C reactive protein (CRP) titer was significantly raised (p<.05) in Anemia of chronic disease and iron deficiency anemia when compared to a group of normal iron status. [Table 3]

Table 1: Demographic and laboratory investigations

Characteristic	IDA (n=46)	ACD (n=17)	NIS (N=9)	p value
Age (years), mean	41.43	43.34	42.46	
Sex;n (%) Female Male	 25 (54.34) 21 (45.66)	 7 (41.17) 10 (58.83)	 3 (33.33) 6 (66.66)	
Hemoglobin (g/dL)	6.94 ±0.145	7.30 ± 0.131	7.32 ±0.16)	
eGFR (mL/min)	28 ±1.2	24.4 ±1.1	26.3 ±1.6	
CRP (mg/dL)	0.40 ±0.01	1.4 ±0.04	1.0 ±0.04	P<0.05
Serum iron(µg/dL)	48.91 ±10.31	56.01 ±10.01	57.82 ±7.61	
TIBC(µg/dL)	178.17 ±9.21	209.30±10.93	197.11±12.42	
TSAT (%)	16.20 ±1.31	26.762 ±.14	29.272 ±.32	P<0.05
Serum ferritin (mg/mL)	108.56±11.01	301.00±21.25	162.63±12.51	P<0.006
Serum hepcidin (ng/mL)	78.71 ±12.06	101.76±26.15	154.81±34.12	P<0.05
Data is shown as mean (SD) unless otherwise specified. ACD, Anemia of chronic disease; CPR, C reactive protein; eGFR, estimated glomerular filtration rate; IDA, iron deficiency anemia; NIS, normal iron status; SE, standard error; TIBC, total iron binding capacity; TSAT, transferrin saturation.				

Table 2: Description of Ferritin according to NKF/KDOQI to bone marrow classification

Groups	S. Ferritin (ng/ml)		
	<100	100-500	>500
IDA (n=46)	28(61%)	16(35%)	02(4%)
ACD (n=17)	02(12%)	06(35%)	09(53%)
NIS (n=09)	04(45%)	03(33%)	02(22%)

Table 3: Description of TSAT according to bone marrow classification

Groups	TSAT		
	<20%	20-50%	>50%
IDA (n=46)	34(74%)	09(20%)	03(6%)
ACD (n=17)	7(41%)	07(41%)	03(18%)
NIS (n=09)	02(22%)	05(56%)	02(22%)

DISCUSSION

Our study tried to establish iron status directly with bone marrow iron reserves and correlate with iron indices and Hepcidin. Iron deficiency, the most common form of Anemia, contributing to 62% of patients. IDA in the setting of CKD with bone marrow iron estimation varies widely, from 20-90%, in different studies.[7,8,20,21] Stancu et al. reported that 48% of subjects have iron-depleted bone marrow in nondialysis CKD.[8] Gotloib et al. found a very high prevalence of IDA in the CKD population with no stainable iron in bone marrow in more than 90% of patients, even after high serum ferritin values. In contrast, Rahman et al. found that only 23% of patients were iron deficient based on the absence of iron staining in bone marrow aspirates.[21,7] We observed serum ferritin was significantly raised in most patients of Anemia of chronic disease (p<0.06), as compared to other groups. This trend was also followed by CRP values with higher values in Anemia of chronic disease; this further supports that inflammatory block could be the probable predominant mechanism involved in Anemia of chronic disease group.

In the IDA group, only 61% of subjects fulfilled the criteria of serum ferritin cutoff value of KDOQI, i.e., less than 100ng/ml. In comparison, 35% of patients had Ferritin between 100-500ng/ml. These values suggest that iron indices are helpful in severe iron-depleted stages, but they cannot be relied upon to diagnose iron deficiency in CKD patients. Our findings corroborate with other studies, suggesting

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that low serum ferritin is a specific marker for IDA in severe iron deficiency. So, a low ferritin level is one of the reliable indicators for iron deficiency anemia, whereas a moderately high serum ferritin value does not exclude iron deficiency.

Mean transferrin saturation (TSAT) values were lower in the Iron deficiency anemia group than in the other 2 study groups. In the IDA group, the mean TSAT value was 16.2%, and 74% of subjects had a TSAT value < 20%, suggesting the high sensitivity of TSAT in IDA. Findings of iron indices differ vastly in studies, which may be due to the remarkable biological variability of TSAT. Stancu et al. show that only 17% of IDA patients had iron indices (TSAT< 20 and S. Ferritin < 100) suggestive of iron deficiency8. Similarly, Rehman et al. studied 52 nondialysis patients. They found that 43 patients had serum ferritin either within or above the therapeutic range, while only nine patients had serum ferritin below 100 ng/ml. On the other hand, in his study, low TSAT (<20%) and high TSAT (>50%) were found in 12 and 9 cases respectively.[7] Marcadal et al. showed that the combined (TSAT-Ferritin) index revealed two main pathologic mechanisms: non-inflammatory ID and hypotransferrinaemia. Absolute ID was uncommon, but its prevalence depended highly on the ferritin cutoff used to define ID.[22]

Most studies mentioned above point to marked variability in iron parameters, which could be partially explained by diurnal changes in serum iron concentration acute-phase reactivity of transferrin in the setting of inflammation, which would lower the TSAT if circulating iron is constant. Transferrin may be low because of decreased synthesis in the setting of malnutrition and chronic disease, which would raise TSAT if circulating iron is constant. There is a wide range of variation in transferrin up to 38%, and therefore, TSAT makes it challenging to interpret this value, more so in CKD, which almost always is accompanied by inflammation and malnutrition.[23]

Serum Hepcidin value was significantly raised in Anemia of normal iron status compared to Anemia of chronic disease. This could be due to Hepcidin playing a predominant role in normal iron status or creating non-inflammatory functional Anemia. In contrast, in Anemia of chronic disease, Ferritin plays a significant role compared to Hepcidin in creating an inflammatory block for Anemia. This assumption is also evident by statistically higher titers of CRP in Anemia of chronic disease and iron deficiency anemia as compared to normal iron status. In CKD, few studies have observed a correlation between Hepcidin and Ferritin; this could be due to multifactorial etiology for raised Hepcidin and non-differentiation between inflammatory or non-inflammatory functional iron deficiency. Zaritsky J et al. have demonstrated that serum hepcidin is raised in CKD. A unique finding in this study was the inverse association between GFR and Hepcidin. The inverse relationship between GFR and Hepcidin reflects the known association between CKD and inflammation.[13] Mercadal et al. showed that Hepcidin was raised in patients with Anemia in CKD with normal iron status and functional iron deficiency but was downregulated in patients with absolute iron deficiency. Only 4% of patients in this study population had absolute iron deficiency, which may be responsible for the lack of absolute significance in this category.[22] H. Goyal et al. showed that hemoglobin decreased with Hepcidin in the absolute iron deficiency group. On the contrary, in patients with functional iron deficiency, Anemia is related to increased hepcidin level.[14]

CONCLUSION

We concluded that serum ferritin, along with CRP, is a good marker for Anemia of chronic disease in CKD but still lacks significance in some of the functional causes of Anemia, where Hepcidin plays a significant role. TSAT value less than 20% is a good marker for IDA but shows an extensive range of variability in the other two groups. Serum hepcidin is one of the emerging molecules, and our study indicated that it has a vital role in functional Anemia apart from the inflammatory block. The present study evaluated anemic, nondialysis-requiring CKD patients who were yet to receive iron supplementation and found serum hepcidin as a helpful marker in differentiating Anemia with adequate iron reserve and Anemia of chronic disease due to inflammation, but these findings are limited to a small cohort. Presently, serum hepcidin is the most promising marker for iron status and needs to be validated in a larger cohort.

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