Correlation of Serum Hepcidin Level with Marker of Inflammation and Iron Status in Chronic Kidney Disease Patients in Chattogram

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Abstract

Background: Hepcidinis a novel biomarker to check the bioavailability of iron among Chronic Kidney Disease (CKD) patients across the world. The study was conducted to evaluate the relationship of serum Hepcidin level with inflammatory markers in CKD patients of stages 3-5.

Materials and methods: This cross-sectional observational study was carried out in the Department of Nephrology of Chittagong Medical College Hospital for a period of one year. Eighty-five conveniently selected CKD patients (Stage 3-5) of whom Haemoglobin (Hb) concentration, iron indices, serum creatinine, C-reactive Protein (CRP) and serum hepcidin were measured. Spearman's rank correlation coefficient was estimated, and simple linear regression was conducted.

Results: Most of the study participants were male, and over 40 years of age. Median serum creatinine was 6.0 (IQR: 3.8-9.9) mg/dl and eGFR was 9.0 (IQR: 6-14) ml/min/1.73m^2. However, serum ferritin was higher among the high hepcidin group (490.5 ng/ml) in comparison to that of the low hepcidin group (218 ng/ml). Similar findings were found for CRP (7 vs. 17) mg/l. A significant positive association of hepcidinwas found with the serum ferritin and a negative association with TIBC and TSAT.

Conclusions: Study results would help to prove the relevance of hepcidinmeasurement among CKD patients in Bangladesh.

Key words: Chronic Kidney Disease; C-reactive protein (CRP); Hepcidin; Hb; Iron profile; Serum creatinine.

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Introduction

The persistent and irreversible disorder known as Chronic Kidney Disease (CKD) is characterized by a progressive loss of kidney function over time, usually caused by diabetes and hypertension.[1] Since 1990, the prevalence of CKD has increased by 29·3% till 2017 with a global prevalence of 9.1%, which constitutes roughly 700 million cases.[2] Bangladesh is also on the list of the rising annual CKD with an overall prevalence of stage-2 to stage-5 renal insufficiency was 24%, 17%, 8%, and 6% respectively.[3]

Anaemia, especially Iron Deficiency Anaemia (IDA) is one of the comorbidities linked to CKD that can significantly affect a patient's quality of life.[4] Commonly, poor iron metabolism and decreased erythropoietin production by the kidneys are cited as causes ofanaemia in CKD.[5] Recent studies, however, have emphasized the part played by chronic inflammation in the onset and progression of anaemia in CKD patients.[4,6,7] It has been demonstrated that increased levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6), limit erythropoietin synthesis and decrease iron availability for erythropoiesis.[4,6]

Several biomarkers of iron status have been used in a clinical setting for patients with CKD. Due to the confounding effects of the acute-phase response on the interpretation of most iron indicators, the assessment of iron indices like serum iron, transferrin, and serum ferritin is challenging when concomitant inflammation is present.[8] In this circumstance, hepcidin, a crucial

iron homeostasis regulator and reduces the amount of iron available for erythropoiesis by inhibiting iron absorption from the stomach and encouraging iron sequestration inside cells, has been emerging as a possible anaemia biomarker in CKD patients.[9]Anaemia of chronic disease, which is frequently seen in CKD patients, has been hypothesized as a potential mechanism for the onset of inflammation-induced elevation of hepcidin.

Therefore, It is necessary to look into the connection between serum hepcidin levels, indicators of inflammation, and iron status among the CKD patients given the crucial role of hepcidin in iron metabolism and the probable association between hepcidin and inflammation in CKD patients. By evaluating the relationship between serum hepcidin levels, inflammation-related indicators, and iron status in stage 3-5 CKD patients, an important information gap will be filled, especially in the context of Bangladesh. Hence, we conducted this study with an aim at evaluating the relationship of serum hepcidin level with CRP and iron indices (Serum Ferritin, Total Iron Binding Capacity and Transferrin Saturation).

Materials and methods

We conducted this analytical cross-sectional study in the Department of Nephrology of Chittagong Medical College Hospital (CMCH) from November 2019 through October 2020.

The study population included the non-dialysis adult CKD patients of stages 3-5 of more than 18 years of age with Hb concentration of <13.0g/dl in males and <12.0g/dl in females. We excluded the patients undergoing haemodialysis, kidney transplant recipients, patients with recent history of acute blood loss or blood transfusion, patients with malignancy, and patients with a history of acute infection within the previous four weeks.

We collected data from 85 eligible patients according to our sample size which was calculated considering the correlation coefficient between hepcidin and CRP of 0.3, 95% confidence interval, and 80% of power. These participants were recruited through the convenience sampling method.

Data obtained were compiled in the Microsoft Excel sheet to generate a master sheet. Then they were fed into a computer software package (SPSS, Version 25) for processing and analysis.

Results

Among the 85 study participants, 54 (63.5%) were male, and most of the patients (61.2%) were aged between 41-60 years. About three-fourths (71.8%) had both hypertension and Diabetes. Most of the patients (71.8%) belonged to Stage 5 CKD. Mean BMI of the participants were 22.4 ± 2.7 kg/m^2. The details of these findings are illustrated in Table I.

Table I Demographic and clinical characteristics of the study participants (n=85)

Characteristics	Frequency (n)	Percentage (%)
Gender		
Female	31	36.5
Male	54	63.5
Age (Mean ± SD)	48.4 ± 13.0	
30 years	11	12.9
31-40 years	12	14.1
41-50 years	26	30.6
51-60 years	26	30.6
> 60 years	10	11.8
Etiology		
DM only	2	1.4
HTN only	19	22.3
DM and HTN both	61	71.8
Others	3	7.1
Stages		
Stage 3	4	4.7
Stage 4	20	23.5
Stage 5	61	71.8
Blood Pressure (Mean ± SD)		
Systolic BP (mmHg)	146.8 ± 13.4	Range: 110-190
Diastolic BP (mmHg)	86.9 ± 7.3	Range: 70-110
BMI (Mean ± SD) in kg/m^2	22.40 ± 2.66	Range: 17.4-31.6

Among the study participants, 13 (15.3%) had the Hepcidin level below 81 ng/ml and the rest (64.7%) were in the other group. Serum creatinine levels were high for both low and high hepcidin groups. Similar findings were found for CRP as well. However, serum ferritin was higher among the high hepcidin group (490.5 ng/ml) in comparison to that of the low hepcidin group (218 ng/ml). The differences between TIBC and TSAT were also significant between the groups. The details of these findings are provided in Table II.

Table II Laboratory parameters of the participants for lowand high hepcidin groups [n=85]

Parameters (Median (IQR) / Mean ± SD)	Low Hepcidin (<81 ng/ml), n1=13	High Hepcidin (≥ 81 ng/ml), n2=72	Total (N=85)	p-value*
Creatinine (mg/dl)	5.7 (2.8-8.5)	6.3 (3.8-10.0)	6 (3.8-9.9)	0.458
Serum ferritin (ng/ml)	218 (156-390)	490.5 (312-690.5)	409 (221-533)	0.002
TIBC (µg/dl)	228 (196-237)	202.5 (146-230)	210 (152-230)	0.048
TSAT (%)	20.4 (16-22)	16.7 (14-21)	16.9 (14.5-21)	0.042
C-reactive protein (mg/L)	11 (7-13)	17 (14.5-25)	16 (8-23)	0.052

*p-value has been derived by Mann-Whitey-U test.

About two-thirds (n=54, 65.9%) of our study participants had iron deficiency, of whom 89.2% had high hepcidin levels in their blood.In this study, hepcidin and Serum Ferritin significantly varied between the patients with normal iron and the patients with iron deficiency. We did not find significant differences in TIBC and CRP between normal and iron deficient groups. The findings are shown in Table III.

Table III Hepcidin, Iron indices and CRP in patients with different iron status [n=85]

Parameters (Median and IQR)	Normal	Iron deficiency	p-value*
Hepcidin (ng/ml	97.6 (81.0-175.7)	149.4 (84.7-278.7)	0.001
Ferritin (ng/ml)	231.0 (223.0-533.0)	398.1 (219.5-531.5)	0.034
TIBC (µg/dl)	216.0 (190.0-247.0)	202.5 (136.0-230.0)	0.109
CRP (mg/l)	13.0 (7.0-18.0)	17.0 (11.5-24.5)	0.062
TSAT (%)	22.6 (20.0-38.5)	15.4 (13.0-17.0)	0.000

*p-value has been derived by Mann-Whitey-U test.
While studying correlation, we found a positive correlation between hepcidin and creatinine in the study and it was statistically significant (rho=0.414, p=<0.0321).We also found a significant positive correlation between hepcidinand ferritin (rho= 0.281, p=0.009), and a significant negative correlation between hepcidin and TIBC (rho= -0.202, p=0.023). The correlation between hepcidin and transferrin was not statistically significant. Figure 1 (A-D) shows the correlation of hepcidin with serum creatinine and iron indices.

Figure 1 Relationship of Hepcidin with Serum Creatinine and Iron Indices

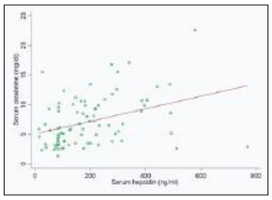

Figure 1-A Hepcidin and S. Creatinine

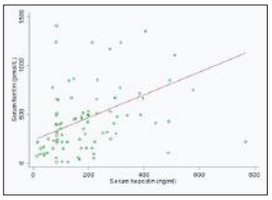

Figure 1-B Hepcidin and S. Ferritin

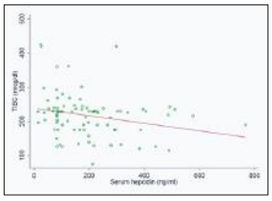

Figure 1-C Hepcidin and TIBC

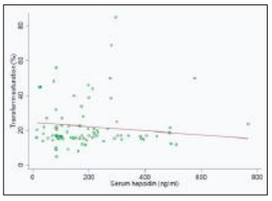

Figure 1-D Hepcidin and TSAT (%)

From the simple regression analysis, we found that hepcidin is positively associated with ferritin, and negatively associated with TIBC and TSAT. With each unit increase of hepcidin, the S. Ferritin would increase by 0.611 unit. On the other hand, with each unit increase of hepcidin TIBC and TSAT would decrease by 0.192 and 0.019 units respectively. We could not document statistically significant association of hepcidin with serum iron and CRP. The findings are shown in Table IV.

Table IV Simple regression analysis between Hepcidin level with iron indices and CRP

Parameters	β coefficients	Standard Error	p-value
Hepcidin and Ferritin			
Ferritin	0.611	0..277	0.03
Hepcidin and TIBC			
TIBC	-0.192	0.051	0.000
Hepcidin and TSAT			
TSAT	-0.019	0.009	0.036
Hepcidin and CRP			
CRP	0.009	0.019	0.612

Discussion

In this analytical cross-sectional study, the mean age of the study participants was 48.36 (±13.04) years and there was male predominance. Similar findings were documented in several research works that looked into hepcidin among the CKD patients.[10,11] In this study, most of the patients had HTN (94.1%) and DM (74.1%) which was along the line of the findings from the study by Bek et al. in 2020.[12] However, a study conducted in 2012

reported higher prevalence of DM among the CKD patients.[13]Most of our patients had stage 5 CKD in the current study. However, a study done by Uehata et al, had most patients with stage 3CKD.[13] This discrepancy may be due to the inclusion of hospitalized patients in our study who belongs to higher CKD stages.

Regarding laboratory parameters, the median serum creatinine was 6.0 mg/dl which was similar to the study by Ali et al.[10] However, the creatinine level in our study was higher than some other studies as well.[12,14] Overall median Serum Ferritin in our study was 409 ng/dl and the same was higher in the high-hepcidin group. This finding echoed the findings from some other studies.[9,15] The median TIBC level in this study was 210 µg/dl which matched with the findings from several other studies,[10,16] and the transferrin saturation or TSAT was 16.9% which was in line with another study conducted in 2018.[10,16,15] TIBC and TSAT, both were increased in the low hepcidin group which mimes a study of 2015 by Rubab et al.[17,18]

Hepcidin was positively correlated with ferritin in this study. Conversely, it was negatively correlated with TIBC and TSAT. These findings are biologically plausible as evident in many studies.[18,19] In addition, hepcidin and ferritin were significantly altered among the patients with iron deficiency in comparison to the patients with normal iron, however, the changes were not documented for either CRP or TIBC. This result had some similarity with that of Sonkar et al. (2019) in which hepcidin, ferritin, and TIBC significantly changed, and there was no effect on iron.[16]

The correlation of hepcidin with serum creatinine was found to be significant which meant that hepcidin was significantly increased with the increment of serum creatinine. This result corresponds with the study of Ali et al.[10] There was also a positive correlation between serum hepcidin and serum ferritin. A similar finding was found in many other studies.[15,20,21] We found a statistically significant negative correlation between hepcidin and TIBC which match with the findings from a study conducted.[21] A negative correlation of hepcidin was documented with TSAT, but it was not statistically significant. Although similar negative correlation was found too in a study,[15]significant positive correlation was found in the study of Mercadel et al.[15,21,]

In this study, simple regression analysis showed hepcidin was significantly associated with ferritin, TIBC, and TSAT (p-value 0.03, 0.000, and 0.036 respectively) but not with CRP. This result matched the study of Sonkar et al.[16] We found a significant relation between hepcidin with ferritin ($p<0.005$) but not with CRP ($p=0.28$). Another study performed by Zaritsky et al. also found significant relation with ferritin ($p=0.001$).[9] Similarly, Uehata et al. revealed significant relation with ferritin ($p<0.001$) and no relation with CRP ($p=0.4$).[13] All these studies including the current study pointed to the significant relation of hepcidin with ferritin while taking all the iron profile parameters into account and no relation with CRP.

Limitation

One of the main limitation of this study was that this was a single centre study. However, the centre is a tertiary level hospital and accommodates referred patients form many hospitals of the region. Secondly, the sample size was relatively small. But, since we did not opt for multivariable analysis, the sample size was sufficient to provide statistical power to our study. Finally, our study will not be generalizable to all CKD patients since we could recruit stage 3-5 patients only.

Conclusion

Our study suggests that serum hepcidin level is related to ferritin, TIBC and TSAT among the non-dialysis CKD patients. Therefore, hepcidin can be a potential biomarker for the iron status of CKD patients. However, since it has been an expensive test, relevant stakeholders and policy makers should take necessary steps to make it available, accessible, and affordable for CKD patients.

Recommendation

The current study reveals that CRP is not an alternative to hepcidin as an inflammatory marker in CKD patients. So, measurement of hepcidin is an essential step for the evaluation of Iron Deficiency Anaemia in non-HD CKD patients. A larger sample size, multicenter study representing all areas of Bangladesh should be done to find a clear picture of our country.

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Contribution of the authors
NN-Concepttion, design, data collection, manuscript writing & final approval.
NUZ- Data collection, drafting & final approval.
SH-Data collection, Data analysis, drafting & final approval.
SS-Data analysis, drafting & final approval.
TJI- Interpretation of data, critical revision & final approval.
MQI-Interpretation of data, drafting & final approval.
MAK-Data analysis, drafting & final approval.
MNH-Study design, critical revision & final approval.
SMUI-Critical revision of content, supervision & final approval.

MSHC-Interpretation of data, critical revision & final approval.

Disclosure
The authors declare no conflict of interest

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