Study of hepcidin level in patients with chronic kidney disease and its correlation with markers of iron status in Zagazig University Hospital

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Context
Chronic kidney disease (CKD) including hemodialysis (HD) is considered an inflammatory state leading to hepcidin upregulation, which affects iron homeostasis.
Aims
The aim was to assess the serum level of hepcidin in CKD including HD patients and its correlation with markers of iron status.
Settings and design
The study was conducted in Zagazig University hospital.
Participants and methods
A prospective, case–control, comparative study was conducted on 66 participants, who were divided into control group, comprising 22 healthy participants; HD group, comprising 22 patients; and CKD group, comprising 22 patients from stages 2 to 4. All participants were evaluated for serum creatinine, complete blood count, estimated glomerular filtration rate by MDRD4 equation, serum iron, total iron-binding capacity, serum ferritin, transferrin saturation, C-reactive protein, and serum hepcidin level.
Statistical analysis
The study was analyzed using SPSS version 20. Description of the qualitative variables was done by frequency and percentage. Description of the quantitative variables was in the form of mean and SD. χ^2-Test, Student's *t*-test, analysis of variance (*F*-test), and correlation analysis were used for analyzing the data.
Results
Serum level of hepcidin was increase by increase stages of CKD, and more increase in regular HD patients. There was high statistically significant positive correlation of hepcidin level with serum ferritin ($P<0.001$), serum iron ($P<0.05$) in CKD and negative correlation with Hb, eGFR ($P<0.001$), and it was not correlated with total iron binding capacity (TIBC) ($P<0.48$).
Conclusion
Serum hepcidin levels are a good biomarker for iron status in patients with HD and CKD. In addition, determination of hepcidin together with markers associated with iron metabolism improves the identification of patients with iron deficiency.

Keywords:
chronic kidney disease, Egypt, Zagazig University, hemodialysis, hepcidin

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Introduction

Anemia is a major complication of patients with chronic kidney disease (CKD) and those with ESRD [1]. Hepcidin, an acute-phase reactant protein produced in the liver, controls the plasma iron concentration by inhibiting iron export by ferroportin from enterocytes and macrophages. Serum hepcidine level was discovered to be increased in those patient as it considered an inflammatory state [2]. Hepcidin leads to erythropoietin (EPO) resistance by regulating iron-restricted erythropoiesis, erythroid progenitor proliferation, and survival [3]. Increased hepcidin across the spectrum of CKD may contribute to abnormal iron regulation and erythropoiesis [4].

Participants and methods

A prospective, case-control comparative study was conducted on adult patients, who were randomly selected from the Nephrology and Dialysis Unit of

Zagazig University hospital, Egypt, from June 2015 to June 2016. This study was conducted on 66 participants who were divided into control group (group I), comprising 22 healthy participant (16 males and six females); hemodialysis (HD) group (group II), comprising 22 patients with mean±SD age of 54.7±6.1 years; and CKD group (group III), comprising 22 patients with CKD with mean±SD age of 51.5±7.4 years. Inclusion criteria were age equal to or more than 18 years. Exclusion criteria included age less than 18 years, patients refusing to participate in the study, patients on immune suppressive therapy, patients with chronic liver disease, patients who had any connective tissue disorders, those with evidence of current infection or inflammation (other than renal cause), patients with malignancy, and pregnant women. An oral consent from the participants and clearance from the institution's ethics committee were obtained for the study.

All persons included in this study were subjected to the following at the time of the study: complete clinical history, physical examination, and laboratory investigation like complete blood count, serum creatinine, serum iron, total iron-binding capacity, serum ferritin, transferrin saturation, C-reactive protein, estimated glomerular filtration rate (eGFR) calculated by the MDRD4 equation, and serum hepcidin level.

Dialysis unit nursing staff performed all blood sample withdrawals on the day of dialysis session for all patients on HD before the intervention and other participants when they were fasting.

Data management

The collected data were revised, verified, and edited on a personal computer, and then analysed statistical using statistical package for the social science (SPSS) for windows, version 20 (SPSS Inc., Chicago, Illinois, USA).

The following statistical tests were used: description of qualitative variables was done by frequency and percentage, and description of quantitative variables was in the form of mean and SD. χ^2-test and Fisher's exact test were used for comparison of qualitative variables with each other. Comparison between quantitative variables was carried out using Student's *t*-test of two independent samples. For comparison of more than two quantitative groups, analysis of variance *F*-test was used for categorical data. Significance level (*P*) was expressed as follows: *P* value greater than 0.05 is not significant, *P* value less than 0.05 is significant, and *P* value less than 0.001 is highly significant. Pearson's correlation coefficient was used to calculate the correlation between quantitative variables. Spearman's coefficient was calculated to determine the relationships between serum hepcidin levels and study variables.

Results

Table 1 showed that there were statistically significant differences between different groups regarding age, whereas there were no statistically significant differences regarding sex.

Table 2 showed that there was a highly significant statistical difference among different groups regarding serum level of Hb, serum iron, total iron binding capacity (TIBC), transferrin, serum ferritin, eGFR, and serum hepcidin.

Tables 3 and 4 showed a highly significant statistical difference among different groups regarding eGFR, Hb, serum ferritin, and serum hepcidin.

Discussion

Normochromic normocytic anemia usually accompanies progressive CKD, with reported prevalence of 47.7% among predialysis patients [5]. Anemia is a major and common complication of CKD that contributes to its progression, cardiovascular events, and high morbidity and mortality in patients with CKD. Renal anemia is considered a special form of anemia of inflammation [6].

Hepcidin levels are regulated by iron status and erythropoietic activity, hepcidin levels are reduced by anemia and hypoxia and increased by

Table 1 Demographic data of different groups

Variables	HD group (*n*=22)	CKD group (*n*=22)	Control group (*n*=22)		*P*
Age (years) (mean±SD)	54.7±6.1	51.5±7.4	50.5±8.	*F*=1.9	0.153 (S)
Sex [*n* (%)]					
Females	6 (21.3)	8 (36.4)	8 (36.4)	χ^2=0.54	0.76 (NS)
Males	16 (72.7)	14 (63.)	14 (63.6)		

CKD, chronic kidney disease; HD, hemodialysis; S, significant.

Table 2 Serum hepcidin and laboratory data of the studied groups

	HD group (*n*=22)	CKD group (*n*=22)	Control group (*n*=22)	*F*	*P*
Hb% (g/dl)	11.14±1.28[#]	11.38±1.24+	14.45±1.18	84.99	<0.001
Serum iron (μg/dl)	80.8±12.07[*,#]	65.0±9.47+	93.18±17.89	23.737	<0.001
TIBC (μg/l)	239.77±42.67[*,#]	290.95±21.52[+]	349.54±51.55	36.274	<0.001
Transferrin sat%	33.54±7.37[*,#]	22.5±4.25+	27.05±6.43	17.866	<0.001
Serum ferritin (ng/ml)	1312.4±801.7[*,#]	141.86±67.55	94.54±32.82	48.43	<0.001
eGFR (ml/min/173 m^2)	6.39±1.89[*,#]	52.72±24.9+	118.04±18.9	211.69	<0.001
Serum hepcidin (ng/ml)	286.61±66.64[*,#]	108.59±30.92	104.13±45.61	95.79	<0.001

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HD, hemodialysis; Hb, hemoglobin; TIBC, total iron binding capacity. *Mean statistical significance when comparing group I with group II. [#]Mean statistical significance when comparing group I with group III. [+]Mean statistical significance when comparing group II with group III.

Table 3 Serum hepcidin and convention markers of iron status between the stages of chronic kidney disease in the chronic kidney disease group

	Stage 2 (*n*=8)	Stage 3 (*n*=8)	Stage 4 (*n*=6)	*F*	*P*
eGFR (ml/min/1.73 m^2)	80.25±7.8[*,#]	47.13±80.59[+]	21.83±4.57	113.94	<0.001
Hb (g/dl)	12.75±0.63[*,#]	11.03±0.69[+]	10.27±0.83	14.78	<0.001
Ferritin (ng/ml)	80.87±33.87[*,#]	184.63±3463[+]	214.16±59.8	17.00	<0.001
Hepcidin (ng/ml)	77.52±17.19[*,#]	110.64±4.94[+]	147.29±15.28	53.82	<0.001

eGFR, estimated glomerular filtration rate; Hb, hemoglobin. *Mean statistical significance when comparing group I with group II. [#]Mean statistical significance when comparing group I with group III. [+]Mean statistical significance when comparing group II with group III.

Table 4 Correlation of hepcidin with the measured parameters in chronic kidney disease and hemodialysis groups

Variables	HD group		CKD group	
	r	*P*	*r*	*P*
Serum ferritin (ng/ml)	0.98	<0.001**	0.91	<0.001**
Serum iron (ng/dl)	–0.15	0.490	–0.41	0.057*
Transferrin saturation %	0.04	0.850	–0.38	0.070
TIBC (μg/dl)	–0.20	0.380	0.15	0.480
eGFR (ml/mn/1072 m^2)	0.19	0.400	–0.95	<0.001**
Hb (g/dl)	–0.95	<0.001**	–0.83	<0.001**

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HD, hemodialysis; Hb, hemoglobin; *r*, correlation coefficient; TIBC, total iron binding capacity. **Correspondence to Correlation is significant at 0.05 (two-tailed). **Correlation is highly significant at 0.01 (two-tailed).

inflammation and intake of food rich in iron without overload [7]. Suppression of hepcidin appears to occur possibly due to the increase of erythropoiesis in response to anemia, which is characteristic of the disease and, consequently, could lead to increased intestinal absorption of iron [8].

The present study revealed that serum hepcidin level was significantly higher in HD group than in CKD groups and control group ($P<0.001$), and also hepcidin was higher in CKD group than control group. Regarding CKD group, there was a significant increase in serum hepcidin level in stage 4 than stage 3, which was higher than stage 2 ($P<0.001$).

Our study agrees with the study of Peters *et al.* [9] who exhibited significantly higher hepcidin-25 levels in patients on HD than patients with CKD, and serum hepcidin levels were decreased slightly during HD. This is owing to contracted levels and underestimation of hepcidin-25 cleanness.

Our finding is consistent with prior studies, which found elevated levels of hepcidin among patients with ESRN [10]. In addition, hepcidin levels were approximately two–three-fold higher in patients with ESRD than in the control group [11]. This was because of limited hepcidin excretion in urine, tissue iron overload, and inflammation [12].

This study revealed that there is a negative correlation between serum hepcidin level and the eGFR in CKD group, whereas this correlation is lost in HD group because the kidney function is completely lost in patients on HD. This result is in agreement with Zaritsky *et al.* [4], who reported that total serum hepcidin (sum of all isoforms 25, 22, 20, and 31) measured with an enzyme-linked immunosorbent

assay was inversely associated with eGFR. This is because hepcidin is excreted in urine and metabolized by the kidney [11]. The impairment of one or both processes may cause hepcidin accumulation as GFR decreases. It remains possible that the inverse relationship of GFR and hepcidin reflects the known association of CKD and inflammation.

We found decreased levels of serum iron, TIBC, and transferrin saturation (TSAT). However, serum ferritin levels were found to be elevated in patients with CKD and HD compared with the control group. High ferritin levels may be observed in this disease owing to functional iron deficiency and reticule-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 the better iron status of the patient [13]. Various other studies also support that current markers of iron metabolism like TSAT and ferritin do not predict iron status effectively [14], and these conventional markers have certain limitations [15]. 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 determination of hepcidin concentration together with conventional markers that are associated with iron metabolism improved the identification of patients with iron deficiency by 26.1% [16].

Our finding revealed a positive correlation between serum hepcidin and serum ferritin level, but there was no correlation between serum hepcidin and serum iron in HD group. Our results were in concordance with Peters *et al.* [9], who reported that after studying of serum hepcidin-25 levels independent of eGFR in 83 patients with CKD not requiring dialysis and in 48 HD patients, serum hepcidin 25 levels were strongly positively correlated with ferritin levels in patients with CKD and HD. In addition, we found there was a negative correlation between serum hepcidin and eGFR, and this was because of inflammation and impaired renal clearance. This was in agreement with Atkinson *et al.* [17] who reported that hepcidin levels correlated negatively with GFR and positively with serum ferritin.The present study showed that serum hepcidin levels have the same sensitivity of serum ferritin, and both have greater sensitivity than transferrin saturation; however, hepcidin specificity was the least in comparison with serum ferritin and TSAt whereas transferrin saturation has the greatest.

Our result showed that there is a negative correlation between serum hepcidin and hemoglobin in patients with HD and CKD as hepcidin levels increased as CKD progressed through stage 3–5. This is similar to Goyal *et al.* [18] who stated hepcidin correlated negatively with Hb in patients with CKD with normal iron status.

In conclusion, serum hepcidin levels are a good biomarker for iron status in patients with HD and CKD. In addition, determination of hepcidin together with markers associated with iron metabolism improves the identification of patients with iron deficiency.

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Conflicts of interest

There are no conflicts of interest.

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