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Serum hepcidin levels as indicator of anemia status in male hemodialysis patients

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***Abstract*---**Background**:** Hepcidin is a polypeptide hormone as regulator of iron homeostasis, and it plays an important role of functional iron deficiency in patients with chronic kidney disease (CKD) which associated with hemodialysis (HD) because of defective erythropoiesis in CKD. This study was conducted to assess the association between hepcidin and anemia status in HD patients. Methods: This was a cross-sectional study of 65 HD patients and EPO therapy were enrolled; compared with 25 age matched healthy individuals as controls. The current study was investigated serum hepcidin, iron status was included serum ferritin and iron levels, anemia hematological indices such as RBC, Hb, MCV, and MCH, in addition to other clinical parameters of kidney function, and C-reactive protein (CRP) as inflammation markers were performed in both HD patients and controls. Results: The mean patient age was (45.65±13.34) years, with dialysis vintage of median (IQR) was (12 (3-36) month. serum hepcidin were highly significantly increase in HD patients than in the control group (622.3±139.6, and 465.16±80.31pg/ml, respectively p = 0.0001). serum hepcidin significantly associated with 37% anemia severity about (692.4±119.8) pg/ml more than patient with 49.2% of moderate about (605.3±117.7) pg/ml, (p=0.007) as compared with 13.8% of mild anemia groups (531.8±99.12) pg/ml, (p=0.001). A negatively significant between hepcidin with dialysis vintage (r= -0.351, p=0.002), and GFR (r= -0.321, p=0.002), while a significant positive strong correlated was observed with ferritin (r= 0.620, p=0.0001), and CRP (r= 0.551, p=0.0001), but its negatively with Iron (r= -0.51, p=0.0001), RBC (r= -0.20, p=0.012), Hb (r= -0.47, p=0.0001), MCH (r= -0.37, p=0.003), and no significant correlated with MCH (r= 0.05, p=0.711). This study concludes that hepcidin was associated with iron status for advanced the pathophysiology of anemia severity in HD patients.

Keywords---hepcidin, iron status, anemia severity, hemodialysis, kidney failure.

Introduction

Anemia is the important complications that associated with chronic kidney disease (CKD) (Stauffer, 2014, Islam et al; 2021). Moreover, anemia is more severe in patients with advanced kidney disease (Ryu et al; 2017). Because anemia increases the risk of patients which undergoing maintenance hemodialysis (HD) cardiovascular disease, and mortality in CKD patients. The important factors that contribute of anemia were Erythropoietin insufficiency, iron deficiency, blood loss, dialysis, chronic inflammation, and conditions that negatively influence erythropoiesis and erythrocytes synthesis in the bone marrow (Webster et al; 2017). Iron homeostasis is kept by absorption of nutritional iron through small intestinal, HD patients increased blood loss either through gastrointestinal duct or due to pathogenic causes can lead to dysregulated of iron metabolism (Ghahramanfard et al; 2019). Hepcidin, a polypeptide hormone primarily synthesis in the liver, it is a play important role to regulate of iron homeostasis in the body (Camaschella et al; 2020). Hepcidin maintains plasma iron levels by reducing ferroportin iron export from enterocytes and macrophages. As a result of increased hepcidin synthesis, plasma iron concentrations fall and erythropoiesis becomes iron-restricted. Hepcidin expression is stimulated by iron overload, inflammation, and erythropoietic activity (Nemeth et al; 2021). Instead of conventional iron indices, Hepcidin, may have been a superior biomarker of anemia in HD patients because it is more fundamental; It reduces accessible blood iron levels by reducing iron export from whole-body iron storage (Lee et al; 2017, Agarwal, 2021). Few studies have examined at the varied relationships between hepcidin and iron indices with anemia severity depending on the anemia and kidney function. Because kidney function affects serum hepcidin levels, the aim of this study to investigate the associations between hepcidin and anemia severity and it was more than anemia associated with iron indices in HD patients.

Materials and Methods

Study design

The current cross-sectional study was performed from January 2022 to march 2022. Conducted 65 patients were ≥18 years of age and had a history of CKD recruited from the center kidney diseases at Al-Sadder Teaching Hospital in Al–Najaf City, Iraq, and 25 age matched healthy individuals as controls the only inclusion criterion was dialysis from first vintage to two times a week. The exclusion criteria were liver disease, all types of malignancy, kidney transplantation, receiving blood in the last 6 months, history of sustained heart disease, infection, inflammatory disease.

The objectives for this investigation, as well as the informed consent form provided from all individuals, were approved by the hospital's institutional review board, and the study was conducted in accordance with the principles of the Helsinki Declaration. The patients were stopped iron supplementation intake for

one weeks before the study. Before dialysis, blood samples were taken to avoid interactions between hemodialysis and hepcidin. Five ml of blood samples were drawn from Fasted participant, and put in tubes with anticoagulant and other jell tubes without any anticoagulant for carefully supernatant serum. By centrifugation at temperature 37 c^o for 10 minutes at 3000 rpm, serum was separated, and frizzed at -80 c^o. kidney function parameters, blood glucose, iron indicis (iron and ferritin), RBC indicis (RBCs, Hb, MCV, and MCH), C-reactive protein (CRP) by automated analysis device, and Hepcidin was measured with ELISA according kit that supply from company.

Statistical analysis

All data were statistically analyzed with IBM SPSS 28 (SPSS Inc., Chicago, IL). the normal distribution used of Kolmogorov-Smirnov test and independent t-test between two groups, ANOVA test and post hoc for comparison among groups. Median (interquartile range) for continuous variables with non-normal distribution. Pearson's correlation tests between hepcidin and studied parameters. Numerical data were expressed as mean ± standard deviation (SD), Nominal data reported by frequencies and percentage with used of chi-square test. The significant level was considered as p-value <0.05.

Results

Demographic and clinical characteristics of Hemodialysis (HD) patients and the control groups

The statistical analysis in the Table (1) indicated to 90 samples consisted of 65 HD patients with median (IQR) dialysis vintage was (12 (3-36) month, compared with 25 healthy participates as control group. No significant differences in the mean ages of the HD patients were (45.65±13.34) compared to control groups were (50±12.73) year, (p=0.164). The difference was not statistically significant (p = 0.081) in mean of BMI HD patients compared with the control groups were (26.88±4.82), and (28.84±4.44) kg/m^2, respectively. Dialysis vintage time of HD patient showed at first D.v. dialysis 11(16.9%), 25(38.5%) were ≤ 1 years, 24(36.9%) ≤ 5 years, and 5(7.7%) were dialysis > 5 years. In the current study, a significant difference (p=0.002) in anemia status proportion of moderate 32(49.2%) and severe anemia 24(37.0%) were more than mild anemia 9(13.8%).

Furthermore, in the same table were parameters of the kidney function, the statistical analysis indicated to high significantly (p=0.0001) increase in Urea (173.54±51.23), and Creatinine (10.14±2.47), and decreased mean of GFR (14.52±10.35) in HD patients as compared with control groups (26.84±4.35) mg/dl, (0.72±0.12) mg/dl, and (109.48±12.64) ml/min/1.73 m^2, respectively. A significantly decreased in mean of Ca in HD (7.08±1.49) to compared with control group (9.1±0.64) mg/dl, p=0.0001, but there were increased in PO levels (4.86±1.26 vs. 3.58±0.51, p=0.0001). A Highly significant difference between HD and controls group in means level of B. Glucose (244.47±61.3 vs. 95.16±12.88, p=0.0001) mg/dl, but decreased in AST (12.97±9.77 vs. 20.89±10.38) IU/L, and ALT (13.81±5.98 vs. 19.98±10.08) IU/L (p=0.001), respectively. Results of inflammation and iron status in HD patients indicated to significant (0.0001)

elevated in means serum of CRP (6.06±1.70), and Ferritin (310.35±103.45) more than (2.20±0.75) mg/l, (74.36±17.54) ng/ml in control groups respectively, but these patients were lowered in means of Iron (52.25±7.35), and Vt.D (12.34±9.41) as compared with (68.03±11.22), and (40.11±7.5) ng/ml. The results of the anemia status were showed significant differences ($p<0.05$) in hematological parameters of HD patients lower than control groups in mean of Hb (8.87±1.92 vs. 13.99±1.41, p=0.0001) mg/dl, RBCs (3.39±0.55 vs. 4.55±0.47, p=0.0001) 10^6/ml, and MCV (86.77±2.39 vs. 88.68±4.86, p=0.015) fL, While the mean of MCH was no significantly different between them groups (31.23±1.89 vs. 30.78±2.25, p=0.347) pg, respectively.

Serum Hepcidin levels

The results showed that HD patients have a high significant (p=0.0001) increase in mean of serum Hepcidin level of (622.3±139.6) pg/ml, as compared with the controls group about (465.16±80.31) pg/ml, Figure (1). Furthermore, hepcidin levels exhibited a significant ($p< 0.05$) increase in 37% of HD patients with severe anemia about (692.4±119.8) pg/ml more than patient with 49.2% of moderate about (605.3±117.7) pg/ml, (p=0.007) as compared with 13.8% of mild anemia groups (531.8±99.12) pg/ml, (p=0.001), but no significant between mild and moderate anemia groups (p=0.099), Figure (2).

Hepcidin associated with Anemia indices and Iron status

The correlation of serum hepcidin levels with demographic and clinical characteristics that studied in HD patients were observed no significant correlation between of hepcidin levels and age (r = 0.118, p= 0.351), and BMI (r= 0.128, p=0.309). A negatively significant between hepcidin with dialysis vintage (r= -0.351, p=0.002), and GFR (r= -0.321, p=0.002), but it's strong positively correlated with CRP (r= 0.551, p=0.0001), table (2). The results observed significantly strong positive correlated ($p<0.05$) of hepcidin with ferritin (r= 0.620, p=0.0001), while its negatively with Iron (r= -0.51, p=0.0001), RBC (r= -0.20, p=0.012), Hb (r= -0.47, p=0.0001), And MCH (r= -0.37, p=0.003), but no significant correlated with MCH (r= 0.05, p=0.711), Figure 3. A, B, C, D, E, and F respectively.

Table 1: Comparison in Demographic and clinical characteristics of Hemodialysis (HD) patients and the control groups

Variables	Mean±SD		p-value
	HD patients n=65	Control group n=25	
Age (year)	45.65±13.34	50±12.73	0.164
BMI (kg/m^2)	26.88±4.82	28.84±4.44	0.081
Dialysis vintage (month)	12 (3-36) #	0.00±0.00	N. A
First D.v.	11(16.9%)	0.00	0.0001
≤ 1 years	25(38.5%)*	0.00	
≤ 5 years	24(36.9%)	0.00	
> 5 years	5(7.7%)	0.00	

Anemia status	Mild	9 (13.8%)	0.00	0.002
	Moderate	32 (49.2%) *	0.00	
	Severe	24 (37.0%)	0.00	
Urea (mg/dl)		173.54±51.23 *	26.84±4.35	0.0001
Creatinine (mg/dl)		10.14±2.47 *	0.72±0.12	0.0001
GFR (ml/min/1.73 m^2)		14.52±10.35 *	109.48±12.64	0.0001
Ca (mg/dl)		7.08±1.49 *	9.1±0.64	0.0001
PO (mg/dl)		4.86±1.26 *	3.58±0.51	0.0001
B. Glucose (mg/dl)		244.47±61.3 *	95.16±12.88	0.0001
AST (IU/L)		12.97±9.77 *	20.89±10.38	0.001
ALT (IU/L)		13.81±5.98 *	19.98±10.08	0.001
CRP (mg/l)		6.06±1.70 *	2.20±0.75	0.0001
Ferritin (ng/ml)		310.4±103.45 *	74.36±17.54	0.0001
Iron (mg/l)		68.03±11.22 *	52.25±7.35	0.0001
Vit. D (ng/ml)		12.34±9.41 *	40.11±7.5	0.0001
Hb (mg/dl)		8.87±1.92 *	13.99±1.41	0.0001
RBC (X10^6/ ml)		3.39±0.55 *	4.55±0.47	0.0001
MCV (fL)		86.77±2.39 *	88.68±4.86	0.0151
MCH (pg)		31.23±1.89 *	30.78±2.25	0.347

Values are expressed as mean ±SD, or # median (IQR) inter quarter range. * Significant differences at p-value <0.05. N.A: non applicated; BMI: body max index; D.v. Dialysis vintage; GFR: glomerular filtration rate; Ca: calcium; PO: phosphor; AST: aspartate aminotransferase; ALT, alanine aminotransferase; CRP: C-reactive protein. Vt.D: vitamin D; RBC: red blood corpuscular; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean concentration hemoglobin.

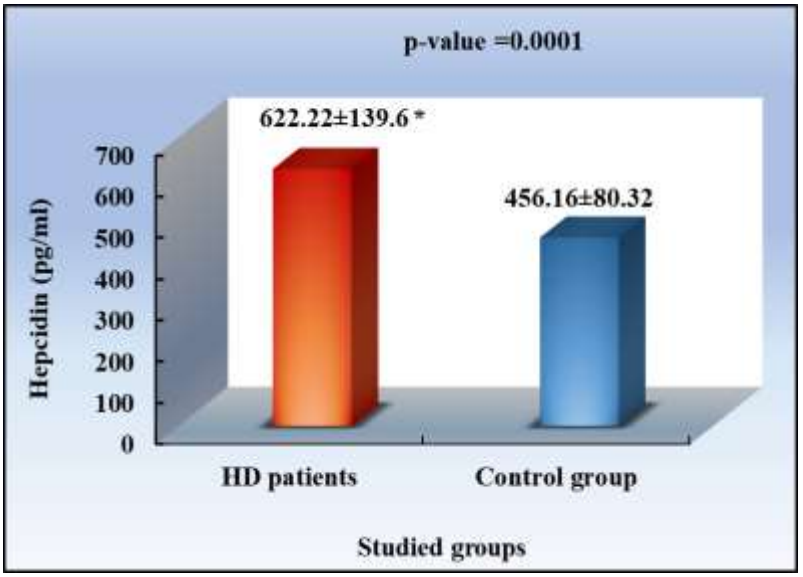

Figure 1. Serum Hepcidin level in HD patients and controls group

Values are expressed as mean ±SD, * Significant differences at p-value <0.05. HD patients n=65, and control n=25.

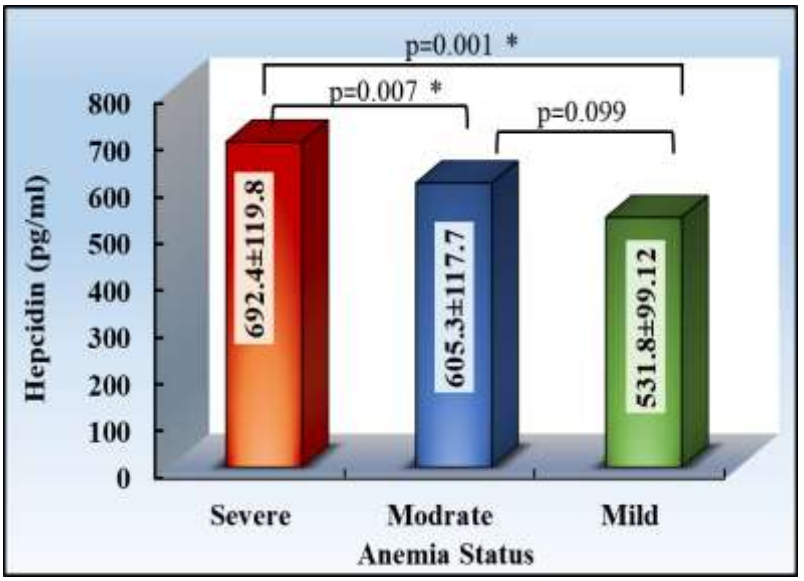

Figure 2. Serum Hepcidin level in HD patients according anemia status

Values are expressed as mean ±SD, * Significant differences at p-value <0.05. Severe n=24, Moderate n=32, Mild n=9

Table 2: Correlation of hepcidin with demographic and clinical characteristics in HD patients

Variables	Hepcidin (pg/ml)	
	r	p-value
Age (year)	0.118	0.351
BMI (kg/m^2)	0.128	0.309
Dialysis vintage (month)	-0.351	0.002
GFR (ml/min/1.73 m^2)	-0.321*	0.009
CRP (mg/l)	0.551*	0.0001

*Significant correlation at p-value <0.05. BMI: body max index; GFR: glomerular filtration rate; CRP: C-reactive protein

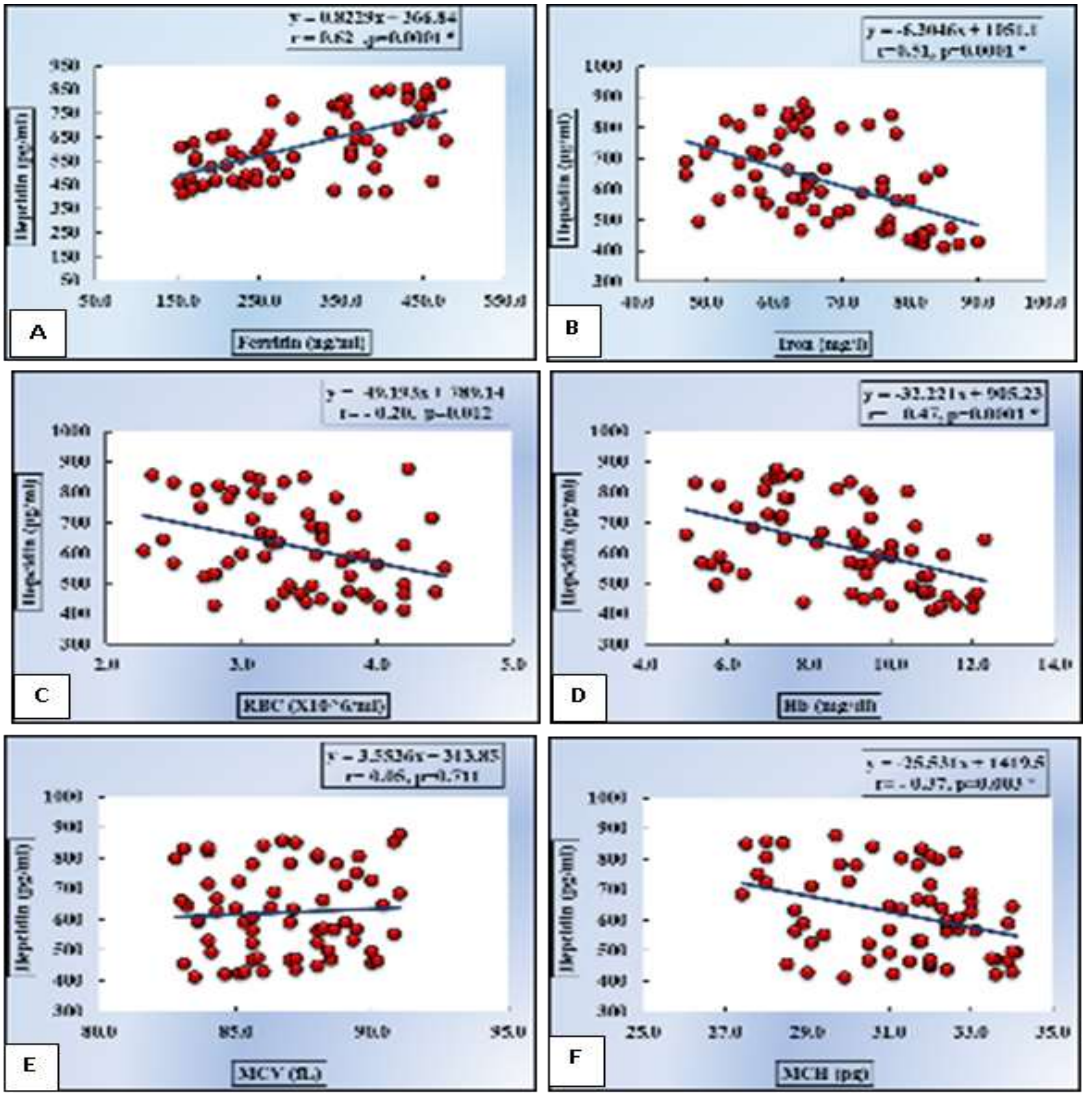

Figure 3. Correlation between serum hepcidin levels with iron status and anemia indices A: positive correlated with ferritin; B: negative correlated with iron; C: negative correlated with RBC; D: negative correlated with Hb; E: no correlated with MCV; F: negative correlated with MCH.

Discussion

In the current study, serum hepcidin level was assessed in 65 HD patients and compared with 25 healthy controls group. According to the results, there were high significant differences in HD patients, and it was associated with anemia severity, in addition to a significant relationship between hepcidin levels and the GFR, CRP, iron status (iron and ferritin), and indicis of anemia status (RBC, Hb, MCV). These founding which was in accordance with many studies concerning elevation of serum hepcidin levels in chronic kidney disease (CKD) on hemodialysis (El Sewefyet al; 2019, Tufekci and Kara, 2021, Hong et al; 2022).

Hepcidin was a regulator of circulation and cellular iron metabolism and release by induced internalization and destruction of ferroportin in the cell's membrane for enterocytes and macrophages, then excess of iron storage in the intracellular (Ganz, 2021, Nemeth, et al; 2021).

According of study conducted by (Eguchi, et al; 2012), and a recent study of Hong et al; (2022) reported that serum hepcidin levels in the early stages or predialysis CKD patients might increase as kidney function declined GFR and hepcidin efflux was blocked, resulting the more severe hepcidin accumulated in the body; lead to serum iron deficient or intracellular iron excess then steadily rise as the disease progressed and renal anemia. As a result, serum iron indicators and hepcidin levels have a significant relationship between them. In the present study showed serum hepcidin was highly correlated with ferritin and relationship with inflammatory marker CRP these results agree with (Zou et al; 2021). Most patients of CKD were in a microinflammation condition, and inflammation raises hepcidin levels, impairing iron intake and utilization in patients, and the inflammatory condition can exacerbate hepcidin deposition (Malyszko et al;2019). Moreover, the current results observed serum hepcidin associated with 37% of HD patients of severe anemia more than patient with 49.2% of moderate anemia ($p=0.007$), these coordinate with (Lee ET AL; 2017), which concluded that Serum hepcidin was associated with anemia severity in progressive CKD patients. Erythropoietin deficiency is the most common cause of anemia in HD patients (Webster et al; 2017, Joksimovic et al; 2022). It was synthesis and release by kidney interstitial fibroblasts and promotes the production of RBCs in the bone marrow. As the CKD advances, erythropoietin levels become insufficient for the severity of anemia, and normocytic, normochromic, and hypo-proliferative anemia might emerge (Webster et al; 2017, Fishbane and Spinowitz, 2018). Studies have attempted to regulate hemoglobin (Hb) levels by supplementing erythropoietin to treat erythropoietin deficiency, but they ultimately unsuccessful to enhance clinical results because erythropoietin resistance is occurring (Lu et al; 2020, Joksimovic et al; 2022). Iron metabolism, Transferrin saturation (TSAT) and ferritin have typically been utilized as serum iron markers, and inflammation cusses increased hepcidin has recently been developed as another factor leading to anemia in HD patients (Gluba-Brzózka et al; 2020).

Conclusion

This was concluded hepcidin was associated with iron status for advanced the pathophysiology of anemia severity in HD patients.

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