Plasma Hepcidin Level in Hemodialysis Patients and its Relationship to Anemia Therapy and Dialysis Efficiency

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ABSTRACT

Background: Hepcidin is a small peptide produced mainly by hepatocytes, macrophages and other cells. In addition to its antimicrobial effects, it is the primary regulator of iron metabolism as it controls both dietary iron absorption from the duodenum and the release of that iron by reticuloendothelial cells.

Objective: Study of plasma hepcidin level and its relationship to the parameters of iron status, anemia therapy, and parameters of dialysis efficiency in hemodialysis patient.

Patients and Methods: 106 subjects were enrolled in our study. They were divided into two groups: **Group 1 (control group)** included 10 healthy subjects. **Group 2** included 96 end-stage renal disease (ESRD) patients on regular hemodialysis; they were subdivided into two groups according to the median plasma hepcidin level. full history, physical examination and detailed analysis of medical records to elucidate the cause of renal failure if present and duration of hemodialysis, estimation of glomerular filtration rate (eGFR) and calculating Kt/V. Laboratory investigations including CBC, liver function tests, kidney function tests, serum iron, serum ferritin, total iron binding capacity (TIBC), transferrin saturation and plasma hepcidin level were measured. In addition to Pelvi-abdominal ultrasound was done.

Results: Plasma hepcidin levels were higher in maintenance hemodialysis patients than in healthy control subjects. Also, there was a significant and independent positive correlation between hepcidin and both serum ferritin levels and KT/V. While there was significant negative correlation between serum hepcidin levels & EPO dose.

Conclusion: High plasma hepcidin levels in HD patients depending on the magnitude of the inflammatory process and on rhEPO doses. Close interaction between hematological indices, iron status and plasma hepcidin levels.

Keywords: Renal failure, Anemia, Iron, Hepcidin.

INTRODUCTION

The hepcidin molecule ("hep" originate from the liver, "cidin" antimicrobial) was described in year 2000 as an antimicrobial peptide that participate in innate immunity.

Initially it was called liver-expressed antimicrobial peptide (LEAP-1), it was isolated from human blood ultrafiltrate and characterized by mass spectrometry as a cysteine-rich peptide synthesized in the liver. Subsequently, it was purified and isolated from urine in its active form [(1, 2)].

The relationship between hepcidin and iron metabolism, at the start, was demonstrated in knockout mice, and these animals exhibited iron overload in contrast to transgenic animals for hepcidin, in which the iron concentration resulted from the consequent development of severe microcytic hypochromic anemia [(3, 4)].

HAMP gene is that gene encoding Hepcidin[(2)]. Stimuli such as iron levels, hypoxia, erythropoiesis, and inflammation control the expression of that gene. In case of anaemia of chronic disease, gene expression increases [(5)]. Several studies have declared the influence of the serum hepcidin concentration on the pathogenesis of hematological diseases such as juvenile hereditary haemochromatosis, in which HAMP gene polymorphism led to hepcidin synthesis deficiency with iron accumulation in the tissues [(6, 7)]. The HAMP gene present on chromosome 19q13 is responsible for hepcidin mRNA transcription through two signaling pathways. The first signaling pathway is related to the bone morphogenetic proteins (BMPs), and the second pathway JAK/STAT (Janus kinase / signal transducer and activator of transcription) signaling pathway, which is related to inflammation [(2)]. BMPs are group of cytokines, which includes transforming growth factor β, however activation of the BMP pathway requires interactions with its cell surface coreceptor haemojuvelin [(8)]. This interaction induces the phosphorylation of the activated BMP receptor, starting an intracellular signaling cascade by binding to a threonine/ serine kinase type I and type II receptor complex [(9)]. The activated type II receptor consequently activates type I receptor, which will transmit the signals to the R-SMAD, SMAD regulatory receiver, induces phosphorylation of SMAD-1, SMAD- 5, and SMAD-8. By this way, a transcription complex involving the SMAD-4 factor is formed [(9)].

Iron is a metal, which is required to maintain several functions in the body such as transport of oxygen, synthesis and repair of DNA, and energetic mitochondrial metabolism, and several enzymes cofactor [(10)]. Hepcidin peptide controls and molecularly regulates iron homeostasis through ferroportin modulation [(1, 2)]. Therefore, that disorder of iron metabolism involving changes of hepcidin and ferroportin relationship causes damage to the body, as these disorders may cause iron overload or deficiency

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[11]. Intracellular excess iron, hyperferraemia, contributes to the production of reactive oxygen species (ROS), causing cell membranes and tissues damages, especially, hepatic, endocrine and cardiac tissues [12]. However, iron deficiency (hypoferraemia) causes decrease hemoglobin synthesis, with the consequent anaemia [13].

Regarding the mechanisms by which hepcidin controls iron homeostasis, it has been shown that it occurs by 3 main mechanisms: inhibition of dietary iron absorption through the duodenum, blockage of iron release recycled by macrophages, and control of the movement of iron stored in hepatocytes [14]. Therefore, systemic iron stores, elevated plasma transferrin concentrations, erythropoietic activity and host defense mechanisms modulate the synthesis of hepcidin [15]. Therefore, when serum iron concentration increases, hepcidin mRNA transcription increases in hepatocytes to decrease iron uptake through duodenal enterocytes and the mobilization of iron stores. On the other hand, when serum iron decreases, hepcidin transcription in the liver will decrease [16, 17].

The kidney is the main clearance organ of hepcidin. In renal failure, there is decrease in the glomerular filtration and excretion of hepcidin and consequently anaemia [18]. Recent studies have shown that elevated concentrations of hepcidin in the serum of patients with chronic kidney disease contribute to increased serum ferritin, decreased iron availability for erythropoiesis, and low hemoglobin levels [19]. Because renal disease is an inflammatory process, the production of IL-1β and IL-6 maintains an activated JAK/STAT pathway with increased hepcidin transcription of the HAMP gene, contributing to anemia via 2 mechanisms: increased inflammation and iron restriction [20–21].

We aimed to study the plasma hepcidin levels and their relationship to the parameters of iron status (serum iron, serum ferritin, TIBC and TSAT), anemia therapy (Erythropoietin), and parameters of dialysis efficiency in hemodialysis patients.

PATIENTS AND METHODS

106 subjects were enrolled in our study. They were divided into two groups: **Group 1 (control group)** included 10 healthy subjects (5 males and 5 females). **Group 2** included 96 (51 males and 45 females) ESRD patients on regular hemodialysis receiving 3 sessions of hemodialysis per week, each session 4 hours using polysulphone membranes, duration of dialysis was more than 6 months and the type of hemodialysis solution is bicarbonate. Dialysis efficiency (single pool Kt/V (spKt/V)) and ultrafiltration volume (mean of the last 3 recorded values) were retrieved from patient's files. The last 3 epoetin and iron doses were recorded, and mean values were used in the analysis. This group was subdivided in to 2 groups according to the median hepcidin level of 300 ng/ml.

Patients with active neoplasm, acute infections, chronic inflammatory disease, any disease causing blood loss, liver or lung disease and patients using anti-inflammatory or immunosuppressive agents all were excluded from our work.

All patients were subjected to thorough medical history taking and full physical examination including blood pressure and anthropometric variables including body mass index (BMI) and waist/hip ratio.

The estimated glomerular filtrate rate (eGFR) was calculated using the modification of diet in renal disease (MDRD) equation: eGFR (mL/min per $1.73\,m^2$) $= 186 * SCr^{-1.154} * Age^{-0.203} * 0.742$ (if female) [22].

Detailed analysis of medical records to elucidate the cause of renal failure if present and duration of hemodialysis (in years).

Complete blood count, liver function tests, serum creatinine, blood urea, eGFR, occult blood in stool to exclude chronic blood loss, iron study (serum iron level, TIBC, transferrin saturation and serum ferritin), pelvi-abdominal ultrasound and measurement of plasma hepcidin level (ELISA).

Measurements and calculations:

The quality of dialysis was assessed during the study period by calculating Kt/V for all the patients. Kt/V is defined as the dialyzer clearance of urea (K, obtained from the manufacturer in mL/min, and periodically measured and verified by the dialysis team) multiplied by the duration of the dialysis treatment (t, in minutes) divided by the volume of distribution of urea in the body (V, in mL), which is approximately equal to the total body water.

Kt/V was calculated according to the Daugirdas second generation formula: Kt/V=ln [R-(0.008 × t)] + [(4 - 3.5R) x (UF/W)], where ln is the natural algorithm, R is the postdialysis/predialysis BUN ratio, t is the dialysis session length in hours, UF is the ultrafiltration volume in liters and W is the post dialysis weight in kg (Range: 0.7 to 2.1) [23].

Ethical approval:

This cross-sectional study was conducted **after approval of the Ethical Committee Board, Zagazig University** and informed written consent from each patient was obtained.

Statistical analysis

All data were reported as mean ± SD. The Data were analyzed by computer using the statistical package SPSS for windows version 16 (software). Comparing means was performed by One-way ANOVA Post HOC LSD test. Correlations between

different parameters were determined by bivariate Pearson correlation test.

The linear relationship between variables was assessed by linear regression analysis. To determine independent factors (Hb concentration, hematocrit, serum levels of transferrin, ferritin and weekly rhEPO dose) affecting hepcidin serum levels, multivariate analysis was done using stepwise logistic regression and odd ratios were estimated for each of the independent variables in the model. For all tests P values ≤ 0.05 were considered statistically significant.

RESULTS

This cross-sectional study was performed on 96 clinically stable hemodialysis patients as well as 10 healthy subjects served as a control group.

As regards the demographic data, age gender and BMI, they showed non-significant differences between studied groups (Table 1).

Table (1): Demographic and anthropometric data of study population:

	Cases	**Control**	**P -value**
Age (years) (Mean ± SD)	45.3 ± 13.17	40.55 ± 8.92	0.116
Gender (% males)	53.13%	50%	0.862
BMI (kg/m²) (Mean ± SD)	22.2150 ± 4.26912	24.6500 ± 4.59147	0.55

Plasma levels of Hepcidin-25 and serum level of ferritin were significantly higher in hemodialysis patients than in controls while serum levels of Iron, TSAT, hemoglobin level and serum albumin were significantly lower in hemodialysis patients than in controls (Table 2).

Table (2): Laboratory findings of the studied groups

	Cases Mean ± SD	**Control Mean ± SD**	**P- value**
Hb (g/dL)	10.25 ± 0.87	13.06 ± 0.88	0.00
Albumin gm/dl	4.13 ± 0.33	4.54 ± 0.35	0.015
Serum Iron (ug/dL)	70.30 ± 4.26	101.65 ± 6.15	0.001
TIBC (ug/dL)	263.42 ± 13.51	291.10 ± 6.20	0.984
TSAT (Percentage)	29.13 ± 2.28	34.38 ± 1.59	0.005
Ferritin (ng/mL)	484.89 ±3.83	140.80 ± 9.84	0.013
Hepcidin-25 (ng/ml)	286.02 ± 11.17	97.05 ± 2.16	0.001

To clarify the demographic, clinical and biochemical characters among the patients' groups in relation to the change in hepcidin level, the median of hepcidin levels in the patients' groups was calculated as 300 ng/ml and patients were divided into 2 subgroups: subgroup 1 with hepcidin < 300 ng/ml and subgroup 2 with hepcidin > 301 ng/ml.

The mean duration of HD was significantly higher in subgroup 2 than in subgroup 1 and the percent of patients on HD > 10 years was 6 % in subgroup 1, while it was 45 % in subgroup 2. The mean concentration of hemoglobin in was significantly higher in subgroup 1 than in subgroup 2. The percent of patients within target hemoglobin > 10.5 g/dL in subgroup 1 was 90 % while it was 10 % in subgroup 2 (Table 3).

Table (3): Demographic, clinical and biochemical characters in all patients and in hepcidin subgroup1 and in subgroup 2

	All (n = 96)	Subgroup 1 (n = 48) Hepcidin<300ng/ml	Subgroup 2 (n =48) Hepcidin>300ng/ml	P-Value
HD Duration (Years) Mean ± SD >10 years	7.60 ± 4.09	5.10 ± 3.21 (6%, 3 patient)	10.10 ± 3.31 (45%, 22 patients)	< 0.001
Hemoglobin (g/dL) Mean ± SD Hb > 10.5 (g/dL)	10.25 ± 0.87	10.75 ± 0.91 (90%, 43 patients)	9.75 ± 0.44 (10%, 5patients)	< 0.001
SerumIron (ug/dL) Mean ± SD	70.30 ± 4.26	64.65 ± 6.91	75.95 ± 2.42	0.14
TIBC(ug/dL) Mean ± SD	263.42 ± 13.51	315.35 ± 38.05	211.50 ± 41.40	0.003
Transferrin saturation Mean ± SD TSAT (20% - 40%) TSAT> 40%	29.13 ± 2.28 .	21.9 ± 1.39 (45%,22 patients) (8%, 4 patient)	36.37 ± 3.55 (71%,34 patients) (31%,15 patients)	< 0.001
Ferritin (ng/mL) Mean ± SD Ferritin (100-800 ng/mL) Ferritin > 800 ng/Ml	484.89 ± 43.83 .	193.87 ± 14.30 (81%,39 patients) (0%)	775.92 ± 61.49 (56%, 27 patient) (45%, 22 patients)	< 0.001
Hepcidin (ng/mL) Mean ± SD	286.02 ± 11.17	200.35 ± 7.54	371.69 ± 8.98	< 0.001
Albumin (g/dL) Mean ± SD Albumin < 4 g/dL	4.13 ± 0.33	4.33 ± 0.31 (15%,7patients)	3.93 ± 0.20 (40%, 19patients)	< 0.001
UF volume (L/session) Mean ± SD	2.99 ± 0.07	3.88 ± 0.55	2.1 ± 0.62	< 0.001
spKt/V Mean ± SD spKt/V < 1.2	1.47 ± 0.29	1.33 ± 0.32 (56%, 27patients)	1.61 ± 0.2 (0%)	0.002
Epoitin alpha dose Patients treated (mg/week) Mean ± SD	4775 ± 158.49	100 % (All) 5800 ± 105.24	100 % (All) 3750 ± 52.19	< 0.001
Iron dose (mg/week) Patients treated Mean ± SD	243.75 ± 8.57	100 % (All) 295 ± 4.68	100 % (All) 192.5 ± 5.44	< 0.001
CRP **Mean ±SD**	3.63 ± 0.8	2.5 ± 0.09	3.3 ± 0.23	0.239

TIBC was significantly higher in subgroup 1 than in subgroup 2. The mean transferrin was significantly higher in subgroup 2 than in subgroup 1 and the percent of patients within target TSAT (20%- 40%) in subgroup 1 was 45 %, while it was 70% in subgroup 2. The percent of patients with TSAT more than the target (> 40%) was 5% in subgroup 1 while it was 30 % in subgroup 2 (Table 3).

Serum level of ferritin was significantly higher in subgroup 2 than in subgroup 1 and the percent of patients within target ferritin (100-800 ng/mL) was 82 % in subgroup 1 while it was 55 % in subgroup 2. The percent of patients with ferritin > 800 ng/mL was 0 % in subgroup 1, while it was 45 % in subgroup 2. The mean plasma level of hepcidin was significantly higher in subgroup 2 than in subgroup 1 (Table 3).

Serum concentration of albumin was significantly higher in subgroup 1 than in subgroup 2. The percent of patients with albumin < 4 g/dL was 15% in subgroup 1, while it was 40 % in subgroup 2. The mean ultrafiltration volume (Litre per session) was significantly higher in subgroup 1 than in subgroup 2. The mean spKt/V was significantly higher in subgroup 2 than in subgroup 1 and the percent of patients with spKt/V < 1.2 was 56 % in subgroup 1 while it was 0 % in subgroup 2 (Table3). The mean required dose of Epoitin alpha was significantly higher in subgroup 1 than in subgroup 2. The mean dose of iron was significantly higher in subgroup 1 than in subgroup 2. CRP levels were non-significant among all groups (Table 3).

Table (4): Correlation of plasma levels of hepcidin with demographic, clinical, and biochemical parameters in the patients

Parameter	R	P
Age (years)	0.146	0.370
Sex (male : female)	0.07	0.93
BMI (kg/m2)	0.830	0.001
Duration of dialysis (years)	0.795	0.001
Ultrafiltration volume (L)	- 0.955	0.001
spKt/V	0.615	0.001
Dose of Epoitin alpha/week	- 0.922	0.001
Dose of Iron	- 0.793	0.001
Albumin (g/dL)	- 0.826	0.001
HB (g/dl)	- 0.783	0.001
Serum Iron (ug/dL)	0.313	.025
TIBC (ug/dL)	0.477	.001
TSAT (Percentage)	0.710	0.001
Ferritin (ng/mL)	0.653	0.00
CRP	0.121	0.247

In simple correlation analysis, plasma hepcidin was correlated positively with TSAT, ferritin, spKt/V, duration of dialysis and BMI, while it was correlated negatively with Hb, dose of epoitin alpha, dose of iron, ultrafiltration volume and serum albumin (Table 4 & Figure 1).

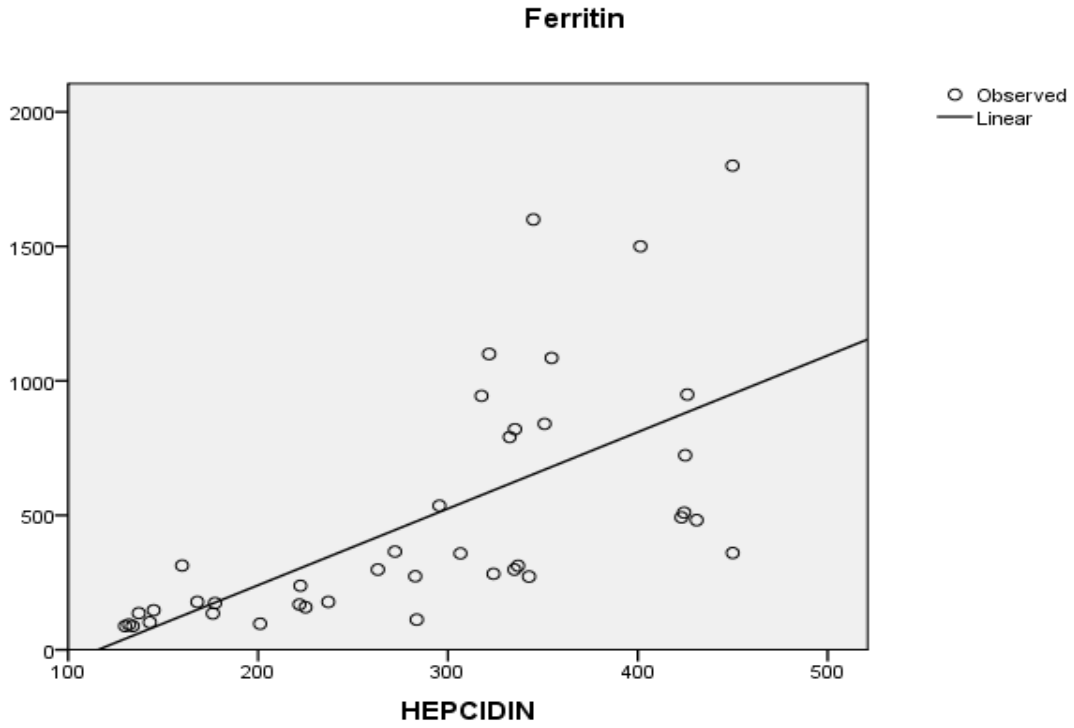

Figure (1): Correlation between plasma hepcidin and transferrin saturation (TSAT) in HD patients

DISCUSSION

Serum hepcidin levels found to be elevated in chronic kidney disease (CKD), this elevation is accused to the development, severity and resistance of anemia to erythropoiesis-stimulating agents [(1)]. Our results demonstrated that serum hepcidin levels were higher in maintenance hemodialysis patients than in healthy control subjects, which is in agreement with **Babitt *et al.*** [(24)] who reported that hepcidin levels are likely to be higher in chronic kidney disease patients due to limited hepcidin excretion and tissue iron overload. The observation that there was a significant and independent correlation between hepcidin, and ferritin levels suggests that it plays a major role in regulating iron homeostasis in this patient group.

In simple correlation analysis in our study, plasma hepcidin was correlated positively with TSAT, and ferritin. The multiple regression analyses also revealed ferritin as significant independent predictor for increased plasma hepcidin.

In our study, a strong correlation was demonstrated between markers of iron storage and serum hepcidin levels in HD patients. This likely reflects the known regulation of hepcidin by iron stores, which was previously demonstrated in populations without CKD [25]. Previous studies that used mass spectrometry method to measure hepcidin also demonstrated a correlation between ferritin and hepcidin in HD patients [26, 27]. In contrast, **Ashby *et al.*** [28] using a radioimmunoassay, did not observe this correlation in HD patients.

In the present study, serum iron and TSAT were not significant predictors of hepcidin levels in multivariate analysis. In contrast to **Kemna *et al.*** [29], who observed that the transferrin concentration regulates hepcidin mRNA expression. On the other hand, a significant correlation between serum ferritin and hepcidin levels is a consistent finding. Serum ferritin is a marker of iron stores in the liver and reticuloendothelial system as well as being an acute phase protein. **Fujita *et al.*** [30] demonstrated that serum ferritin level had a strong positive correlation with the hepatic level of hepcidin mRNA expression.

In the present study, we found that there was significant negative correlation between serum hepcidin levels & erythropoietin (EPO) dose in accordance with the fact that increased erythropoiesis leads to suppression of hepcidin [31]. **Costa *et al.*** [32] reported that erythropoietin down-regulates liver hepcidin expression, hepcidin-inhibitory hormone. The Pathways via which EPO treatment modulates hepcidin levels are largely unknown. Alternatively, EPO administration may indirectly lead to suppression of hepcidin via increased levels of growth differentiation factor-15, which is secreted by erythroblasts [33] or via the molecule named twisted gastrulation (TWSG1). TWSG1 is expressed during erythropoiesis and acts by inhibiting bone morphogenic protein-induced expression of hepcidin [34].

Although, we studied a relatively small and heterogeneous patient population, and our results with respect to the hepcidin decrease in response to EPO are unambiguous. It seems that the signaling pathway that connects EPO with hepcidin is not markedly influenced by systemic disturbances seen in patients with kidney failure.

In our study, we found a positive correlation between KT/V & serum hepcidin level, this finding is supported by the findings of **Zaritsky *et al.*** [35] who did not observe an inverse correlation between hepcidin and Kt/V but this might simply reflect the much larger size of hepcidin when compared with urea.

CONCLUSION AND RECOMMENDATIONS

Close interaction between hematological data, iron status and hepcidin serum levels, which ultimately regulate intracellular iron availability. Hepcidin and its regulatory pathways are potential therapeutic targets, which could lead to effective treatment of anemia of chronic renal failure patients.

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