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Hepcidin in hemodialysis patients and its association with anemia and serum iron indices

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ABSTRACT

Introduction: Anemia is one of the most common complications in dialysis patients. Deficiency of erythropoietin is the most common cause of anemia in dialysis patients. Chronic kidney disease (CKD) patients have a negative iron balance. Hepcidin is an antibacterial peptide that its synthesis is controlled by the iron surface and inflammatory markers and plays an important role in iron hemostasis in hemodialysis patients. In the presence of anemia, the level of hepcidin in normal individuals is reduced. Therefore, the normal level of hepcidin in CKD individuals is considered high due to their anemia level, while, the level of hepcidin is reduced after dialysis.

Objectives: We aimed to study the level of hepcidin-25 in hemodialysis patients and its relationship with anemia and serum iron indices.

Patients and Methods: In a cross-sectional study, 70 patients who fulfilled inclusion criteria enrolled in the study. Serum hepcidin 25 was measured by ELISA method. At the same time, blood samples were taken to measure other indices including iron, ferritin, C-reactive protein (CRP), total iron binding capacity (TIBC) and albumin. Hepcidin-25 levels compared with other mentioned parameters. Statistical analysis was done in SPSS 18.0 software.

Results: No significant relationship between inflammatory cytokines, TIBC and CRP and erythrocyte sedimentation rate (ESR) with hepcidin levels was detected. Additionally, relationship between serum iron, ferritin and hepcidin levels was not significant ($P > 0.05$).

Conclusion: Assessment of hepcidin 25 level for inflammation and iron status does not seem to be helpful for anemia management in hemodialysis patients.

Introduction

Anemia is a common complication in dialysis patients. Among different types of anemia, normocytic normochromic and mild hypochromic anemias, which originate from insufficient erythrocyte production, occur more prevalently in these patients (1,2). Many factors contribute to these anemias, including erythropoietin, iron deficiency, blood loss, dialysis, chronic inflammation, and factors which negatively affect erythropoiesis (3,4). Previous studies have been addressed "erythropoietin" deficiency as the most prevalent cause of anemia in dialysis patients (5,6). Regarding the anemia in patients with chronic kidney disease (CKD), increased blood loss either through gastrointestinal tract or due to pathogenic causes can lead to a negative iron balance, resulting in reduced levels of ferritin, iron as well as iron saturation to less than 16% (7). Decreased intestinal iron absorption in dialysis patients have been clearly reported in some studies (8,9).

Hepcidin is an antibacterial peptide which has been

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regulated by iron and inflammatory markers, and plays a key role in iron hemostasis in dialysis patients. The peptide is produced in the liver and acts as a normal regulator of hemostasis and a negative regulator of iron absorption in normal conditions. High levels of hepcidin inhibit iron absorption from duodenum and iron release from macrophages (10). It is generally accepted that oral iron intake can increase the levels of hepcidin in normal individuals. Whereas, iron deficiency can severely affect the levels of hepcidin in mice (11). Acute type II inflammatory reactants, associated with interleukin 6, are shown to be responsible for hepcidin production. In anemia, erythropoietin and hypoxia resulted in increasing of iron absorption while reducing hepcidin production (12,13). The occurrence of anemia in normal individuals decreases hepcidin levels. Therefore, with regard to the level of anemia, normal levels of hepcidin are considered high in patients with CKD. Moreover, in healthy erythropoietin-receiving individuals, the levels of hepcidin decrease to 70%-75% in 24 hours (14).

Studies have been shown that hepcidin is independently related to creatinine, triglycerides, and the remaining kidney function in dialysis patients. Hepcidin is increased in dialysis patients, but there is no relationship between increased levels of hepcidin and the level of interleukin-6 or unresponsiveness to treatment. However, this increase reverses following treatment with erythropoietin (15). Furthermore, the level of hepcidin decreases after dialysis (16,17). Thus, dialysis and inflammation have different effects on reducing or increasing of hepcidin in dialysis patients due to anemia.

Objectives
This study aimed to determine the level of hepcidin in dialysis patients and its relation with anemia, treatment-resistant anemia, and the levels of ferritin, albumin, and inflammatory factors for finding new therapeutic approaches (anti-hepcidin antibody) regarding treatment of anemia and end-stage renal disease (ESRD) in hemodialysis patients.

Patients and Methods
Patients
This study was conducted on over 18 years old hemodialysis patients in Kowsar hospital of Semnan, Iran. The only inclusion criterion was dialysis from at least three times a month to three times a week. The exclusion criteria were the history of sustained cardiovascular disease, acute infection, active inflammatory disease, pregnancy, receiving blood in the last 6 months, any evidence of active or hidden (internal) bleeding, liver disease, any type of malignancy, and using immunosuppressive drugs. The study objectives were described to patients before enrollment in the study and written informed consent was obtained from all participants. The patients were asked to stop iron compounds 2 weeks before the study. In order to eliminate interaction between hemodialysis and hepcidin, blood samples were collected before dialysis. Hepcidin-25 was measured with ELISA. Other indices including iron, ferritin, albumin, iron saturation, reticulocyte count, and CRP (C-reactive protein) were also measured (Latex agglutination). Finally, the relationship between hepcidin-25 and the mentioned parameters was statistically analyzed.

A total of 2 mL blood was collected from fasted patients in anticoagulant-containing tubes; and serum was separated through retaining them at room temperature for 20 minutes and centrifugation at 3000 rpm for 10 minutes. The samples supernatant (serum) was carefully removed and kept at -80°C. Twenty-four hours before measurement, samples were transferred to 2-8°C. Hepcidine-25 was measured using the ELISA kit.

Ethical issues
Human rights were respected in accordance with the Helsinki Declaration 1975, as revised in 1983. The informed consent was taken from the hemodialysis patients. The study was approved by ethics committee of Semnan University of Medical Sciences (Ethical cod# IR.SEMUMS.REC.1394.116). This article resulted from a general practitioner thesis by Arash Mirzaei (research project # 915) funded by deputy of research, Semnan University of Medical Sciences.

Statistical analysis
The data were statistically analyzed with IBM SPSS 22 (SPSS Inc., Chicago, IL) through Kolmogorov-Smirnov test and t test (or Mann–Whitney U, Pearson's, or Spearman's correlation coefficient tests) and the results were reported as mean ± standard deviation. The significant level was considered as 5%.

Results
Hepcidin-25 was measured in 69 patients admitted for hemodialysis to Kowsar hospital of Semnan, Iran; 58% of them (n = 40) were male and the rest were female. The level of hepcidin-25 was 1486.1 ± 1659.2 pg/dL (with a median of 1361 pg/dL) in males and 1722.9 ± 1821.7 pg/dL (with a median of 1603.7 pg/dL) in females. The results showed no significant difference in the mean of hepcidin-25 between males and females ($P = 0.580$). The mean age of patients was 62.3±14.9 years ranging from 29 to 95 years. No significant correlation was found between the levels of hepcidin-25 and age of patients ($P = 0.604$, r = -0.064). CRP was negative in 78% (n = 54) and positive in 21.7% of the patients (4.3% weak positive, 8.7% positive, 8.8% CRP++). There was no significant difference between CRP-negative and CRP-positive patients regarding hepcidin-25 level distribution ($P = 0.556$).

Total iron binding capacity (TIBC) was normal (250-

400 mcg/dL) in 71% of patients (n = 49), <250 mcg/dL in 2.9%, and >400 mcg/dL in the rest. There was no significant correlation between TIBC and hepcidin levels (*P* = 0.229, *r* = -0.147) (Table 1).

Iron
The level of iron was normal in 53.6% (n = 37) of patients and low in the rest (46.4%). None of the patients had an iron level of higher than normal. There was no significant correlation between serum iron and hepcidin-25 levels (*P* = 0.289, *r* = 0.130; Table 2).

Ferritin
The level of ferritin was in the normal range (20-110 ng/dL in women and 20-250 ng/dL in men) in 30.4% of the patients (n = 21), and high in the rest. There was no significant correlation between ferritin and hepcidin levels (*P* = 0.379, *r* = 0.108; Table 3).

Erythrocyte sedimentation rate
Erythrocyte sedimentation rate (ESR) was in the normal range in 34.8% (n = 24) (<15 h/mm in men and <20 h/mm in women) and abnormal in 65.2% of patients. There was no significant correlation between ESR and hepcidin-25 levels (*P* = 0.060, *r* = 0.228).

Albumin
The level of albumin was normal (3.5-5 g/dL) in 68.1% (n = 47) and <3.5 in 31.9% of patients. There was no significant correlation between serum albumin and hepcidin levels (*P* = 0.367, *r* = 0.110).

Ferritin
According to the results, the level of ferritin was in normal range (20-110 in women and 20-250 in men) in 30.4% (n = 21) of patients and was high in the rest of the patients. There was no significant correlation between serum ferritin and hepcidin levels (*P* = 0.379, *r* = 0.108; Table 3).

Discussion
In this study, hepcidin-25 level was evaluated in 69 hemodialysis patients. According to the results, there was no significant relationship between hepcidin and the levels of inflammatory factors, albumin, iron, ferritin, and TIBC. Moreover, 58% of the subjects were males and the rest were females with a mean hepcidin level of 1486.1. There was no significant difference in the distribution of hepcidin between men and women.

In contrast with our findings, Brătescu et al (18) found that the level of hepcidin was significantly lower in men than women. However, gender has not been considered in other results reported in the literature. Both studies had different results with the present study due to small sample size. Furthermore, no significant relationship was observed between age and hepcidin levels among different age groups in this study.

There was also no significant relationship between inflammatory indicators of CRP and ESR with hepcidin, which was in accordance with the study conducted by Abbasi Larki et al (19). They did not find any association between hepcidin, ESR and CRP. Additionally, Kuragano et al (20) found similar results. Likewise, Kato et al (21) showed that CRP has no relationship with hepcidin. Similar results have been found by van der Putten et al (22).

However, our results were in contrast with the studies of Malyszko et al (23), Uehata et al (24), and Brătescu et al (18). They studied the relationship between hepcidin and inflammation indicators such as CRP. These contradictory results may arise from small sample size of similar studies, which should be addressed in the future studies.

No significant correlation was found between TIBC values and hepcidin levels in the present research. This was consistent with the studies of Uehata et al and Abbasi Larki et al (19,24). They also did not find any association between iron stores and hepcidin (19,24).

There was no significant correlation between levels of

Table 1. Compare of hepcidin levels in patients in term of levels of TIBC

TIBC (mcg/dL)	No. of samples	Mean	Standard deviation	Median	Inter-quartile range	*P* value	Correlation coefficient
<250	2	462.5	67.2	462.5	-		
250-400	49	1652.9	1801.1	650.0	3200	0.229	- 0.147
>400	18	1527.2	1585.0	572.5	3124		

Table 2. Comparing hepcidin levels in patients regarding the level of iron

Iron level	No. of samples	Mean	SD	Median	Inter-quartile range	*P* value	Correlation coefficient
Less than Normal	32	1305.1	1499.7	498.0	1788	0.289	0.130
Normal	37	1828.2	1876.8	695.0	3240		

Table 3. Mean, standard deviation, range and inter-quartile range of hepcidin-25 levels regarding ferritin levels

Ferritin level (ng/dL)	No. of samples	Mean	SD	Median	Inter-quartile range	*P* value	Correlation coefficient
Less than Normal	21	1369.6	1536.9	495.0	2458	0.379	0.108
Normal	48	1680.1	1801.7	665.0	3165		

albumin and hepcidin, which was in accordance with the study reported by Pezeshki et al (19).

In addition, there was no significant relationship between the levels of serum iron and hepcidin, which is consistent with the study of Pezeshki et al (19). However, our findings were in contrast to the study by Malyszko et al. They showed that decreased serum iron can lead to an increase in hepcidin. This inconsistency may arise from the lack of subjects with low serum iron level which can affect levels of hepcidin (23).

Our results showed their similarity with Pezeshki et al, Taheri et al, Uehata et al, Vander Putten et al, Hilde et al, and Tessitore et al, while they could not find any relationship between hepcidin and ferritin (19, 22, 24- 27).

Wagner et al showed that high ferritin levels can affect hepcidin, which seems distinctly different from our research. However, this inconsistency can arise from high sample size in the present study (29). In general, it can be concluded that there was no significant relationship between inflammatory factors, levels of albumin, iron saturation, TIBC, and ferritin with hepcidin.

Conclusion
There was no significant relationship between serum hepcidin-25 level with inflammatory factors, serum albumin level, iron saturation, TIBC, and ferritin in hemodialysis patients. So assessment of hepcidin 25 level for inflammation and iron status does not seem to be helpful for anemia management in hemodialysis patients.

Limitations of the study
A limitation in this study was relatively low sample size. The same study in larger sample size is suggested.

Authors' contribution
AM and MY searched the literature. FGh VS prepared the primary draft. FGh and MY performed first the edition. MY conducted final edition. VS did laboratory tests. RGh performed statistical analysis. All authors wrote and signed the final paper.

Conflicts of interest
The authors declared no competing interests.

Ethical considerations
Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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