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Original Article

Clinical significance of serum hepcidin for the treatment of anemia in maintenance hemodialysis patients

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Abstract: Anemia is a frequent complication in the maintenance hemodialysis (MHD) patients. However, data on serum hepcidin level as a guide in MHD patients are limited. We aimed to investigate the clinical significance of serum hepcidin level on erythropoiesis-stimulating agents (ESAs) and/or intravenous iron therapy among MHD patients. A total of 32 MHD patients receiving ESAs and iron therapy were enrolled; 30 age-and gender-matched healthy subjects were selected as controls. Serum levels of hepcidin, ferritin, iron, transferrin saturation, and erythropoietin were determined. Serum hepcidin level was significantly higher in MHD patients than the control group (424±174.2 ng/mL and 72.4±12.3 ng/mL; $P<0.01$). Serum hepcidin level in MHD patients was positively correlated with serum iron (r=0.68, P=0.005), serum ferritin (r=0.62, P=0.0045), and transferrin saturation (r=0.7, P=0.001). A negative correlation was observed between hepcidin and reticulocyte count (r=-0.63, P=0.015). Moreover, serum hepcidin almost restored to normal level after hemodialysis. EPIAO alone but not intravenous iron sucrose could effectively reduce hepcidin level. In conclusion, serum hepcidin level is increased in MHD patients. Hepcidin may involve in the disturbance of iron metabolism and regulation of erythropoiesis in these patients. Reduction of hepcidin level and sufficient ESAs supplementation can improve erythropoiesis and restore iron homeostasis.

Keywords: Hepcidin, maintenance hemodialysis, iron homeostasis, anemia

Introduction

Anemia is a frequent complication of chronic kidney disease and end-stage renal disease (ESRD). Insufficient production of erythropoietin and iron deficiency is the main cause of anemia in maintenance hemodialysis (MHD) patients. Use of erythropoiesis-stimulating agents (ESAs) has significantly improved the outcome of renal anemia. Unfortunately, a considerable percentage of MHD patients are often suboptimal response to ESAs [1, 2]. Absolute and functional iron deficiency has been considered as the main cause of the hyporesponsiveness to ESAs [3]. Moreover, excessive iron supplementation can aggravate iron overload and subsequently induce oxidative stress [4].

Hepcidin is a novel hepatocyte-derived peptide hormone and plays a crucial role in iron homeostasis. Elevated hepcidin attenuated iron overload through decreasing iron absorption and increasing sequestration within the reticuloendothelial system under the condition of iron repletion [5]. Increased inflammation and decreased clearance of hepcidin can lead to higher serum level of hepcidin in MHD patients [6, 7]. Hepcidin has been suggested as a tool for managing iron therapy in hemodialysis patients on ESAs [8]. However, whether serum hepcidin may help clinicians predicting and monitoring iron therapy in MHD patients remains debating [9, 10].

In the current study, we investigated the clinical significance of serum hepcidin level on ESAs and/or intravenous iron therapy among MHD patients and provided recommendations for iron supplementation in these patients.

Materials and methods

Participants

This study was approved by the ethics committee of Tongji University and written informed consent was obtained from all participants. A total of 32 MHD patients were recruited from

Table 1. Baseline clinical characteristics and laboratory parameters

Variables	MHD patients (n=32)	Healthy controls (n=30)	*P*-value
Age (years)	64.2±11.3	35.2±7.4	P<0.01
Gender (male/female)	19/13	18/12	P>0.01
Time on dialysis (years)	4.0±0.1	-	-
Ultrafiltration volume (L)	2.3±0.7	-	-
Kt/V	1.52±0.3	-	-
Serum iron (μmoL)	18.2±2.1	20.4±4.6	P>0.01
Serum ferritin (μg/L)	456±38.7	138±8.6	P<0.01
Transferrin saturation (%)	37±4	38±3	P>0.01
Reticulocyte (× 10^9)	29.6±4.2	32.4±3.8	P>0.01
Hemoglobin (g/L)	124±1.4	128±1.8	P>0.01
Hs-CRP (mg/dL)	2.7±0.4	2.1±0.3	P<0.05
Hepcidin (ng/mL)	424±174.2	72.4±12.3	P<0.01

Kt/V; K, dialyzer clearance; t, time; V, volume of water contained within the body; Hs-CRP, high-sensitivity C-reactive protein.

the Blood Purification Center of the Affiliated Hospital of Tongji University from October 2013 to June 2015. Of these patients, 19 cases were male and 13 female, with a mean age of 64.2±11.3 years old. The patients were further divided into three subgroups (intravenous iron alone, EPIAO alone, and combination of intravenous iron and EPIAO) depending on their treatment. The underlying diseases in the intravenous iron group were chronic nephritis (*n*=3), hypertensive renal impairment (*n*=2), diabetic nephropathy (*n*=3), obstructive nephropathy (*n*=1), and polycystic kidney (*n*=1). The combination of intravenous iron and EPIAO group included chronic nephritis (*n*=4), diabetic nephropathy (*n*=3), hypertensive renal impairment (*n*=2), IgA nephropathy (*n*=2), and unknown origin (*n*=1). Thirty healthy persons (mean age 35.2±7.4 years; male/female 18:12) with normal serum levels of urea and creatinine were selected as the controls.

Patients satisfying the following inclusion criteria were included: 1) they had renal-related anemia (hemoglobin level ≤110 g/l for women or ≤120 g/l for men); 2) undergone maintenance hemodialysis 2-3 times/week for at least 6 months; and 3) received stable ESAs and iron therapy for 3 weeks or longer prior to the enrolment. Patients were excluded if they had severe liver disease, malignant tumors, active or chronic hemorrhage, intermittent blood transfusion, chronic hypoxic disease, or application of antibiotics owing to severe infection within the last four weeks.

Data collection and measurements

Clinical characteristics and biochemical parameters, including medical history, age, gender, cause of chronic kidney disease, hemoglobin, reticulocyte, serum iron, serum ferritin, transferrin saturation, serum erythropoietin, and high-sensitivity C-reactive protein (hs-CRP) were collected on the enrolment. The estimation of the adequacy of dialysis were calculated using single-pool Kt/V for urea as follows: spKT/V = -ln (R-0.008t) + (4-3.5R) × UF/W. Serum hepcidin level was measured by the enzyme-linked immunosorbent assay (ELISA) using a commercially available kit from American R&B Company before and after dialysis. The levels of hemoglobin, reticulocyte, serum iron, serum ferritin, transferrin saturation, serum erythropoietin, and hs-CRP were determined by the standard laboratory methods.

Treatment

All the MHD patients were divided into a iron sucrose group (Venofer 100 mg/week, Beijing Novartis Pharma Ltd. *n*=10; mean age 65.4±8.2 years), a recombinant human erythropoietin group (EPIAO, *n*=10; mean age 67.2±10.2 years), and a combination of intravenous iron and EPIAO group (Venofer 100 mg/week plus EPIAO; *n*=12; 58.4±10.3 years). The appropriate dose of EPIAO depended on the previous treatment. The EPIAO dose was reduced by 25% when the hemoglobin level reached 110 g/l for the female or 110 g/l for the male. All the patients adopted dialyzer membrane (Weigao products) an area of 1.4 square meters under Gimbo dialyzers. After 2 weeks' treatment, all the serum parameters were determined before hemodialysis.

Data analysis

Continuous data were expressed as mean ± standard deviation (SD). T tests were applied to observe the mean difference between MHD patients and healthy controls. Spearman rank test was applied to investigate correlations

Table 2. Comparison between the different treatment groups

Variables	Iron sucrose (n=10)	EPIAO (n=10)	Iron sucrose + EPIAO (n=12)
Dosage of drugs	Iron sucrose 100 mg/week	512±46 IU/kg/week	Iron sucrose 100 mg/w + 342±52 IU/kg/week
Hepcidin (ng/mL)			
On enrolment	412±168.4	421±172.8	430±178.3
Week 2 after treatment	434±171.3	286.4±94.2#	412±171.4
Hemoglobin (g/L)			
On enrolment	128±3	119±4	123±3
Week 2 after treatment	126±3.2	118±5	122±3
Serum ferritin (μg/L)			
On enrolment	512±87	560±76	620±68
Week 2 after treatment	581±89	512±68	654±74
Reticulocyte (× 10^9)			
On enrolment	31.8±4.2	26.8±3.6	27.6±6.3
Week 2 after treatment	45.6±5.4*	31.4±3.8	44.8±5.6#
Transferrin saturation (%)			
On enrolment	34±8	39±5	38.2±4
Week 2 after treatment	42±7*	38±4.8	36.6±5
Serum erythropoietin (mg/dL)			
On enrolment	9.2±2.8	9.2±2.7	8.3±2.1
Week 2 after treatment	6.7±3.2	68.5±48.6#	19.2±6.2#

*P<0.05; #P<0.01.

between serum hepcidin and other biomarkers. All the statistical analyses were conducted by using SPSS software 16.0. A *P*-value <0.05 was considered statistically significant.

Results

Baseline clinical characteristics

As shown in **Table 1**, in healthy controls, serum ferritin level was 138±8.6 μg/L, while a significantly higher serum ferritin value of 456±38.7 μg/L was observed in MHD patients (P<0.01). Serum hepcidin level of MHD patients was significantly higher than those in the healthy controls (424±174.2 vs. 72.4±12.3 ng/ml, P<0.01). There were no statistically differences between MHD patients and healthy controls with respect to serum iron, transferrin saturation, reticulocyte, hemoglobin, and CRP level (all P>0.05). Hemodialysis significantly decreased serum hepcidin level (424±174.2 vs. 72.4±12.3 ng/ml, P<0.01). Spearman rank analysest indicated that serum hepcidin showed significant positive correlation with serum iron (r=0.68), serum ferritin (r=0.62), transferrin saturation (r=0.70), and reticulocyte (r=-0.63). However, there were no significant correlation between serum hepcidin level and hs-CRP, hemoglobin or spKT/V.

Changes of serum hepcidin after treatment

After two weeks' treatment, we detected serum hepcidin, hemoglobin, reticulocyte, transferrin saturation, serum ferritin, and serum erythropoietin level before hemodialysis. Treatment with intravenous iron sucrose alone, serum hepcidin level was slightly increased and serum erythropoietin level showed no obvious reduction (all P>0.05); while reticulocyte and transferrin saturation level significantly increased (all P<0.05). EPIAO treatment alone significantly increased serum erythropoietin level and decreased serum hepcidin level (all P<0.01). Treatment with iron sucrose combined with EPIAO significantly increased serum erythropoietin and reticulocyte level (all P<0.01) but not significantly reduced serum hepcidin level (P>0.05) (**Table 2**).

Discussion

This study reveals that serum hepcidin level is higher in MHD patients than the healthy controls. Hepcidin level in MHD patients is positively correlated with serum iron, serum ferritin, and transferrin saturation, negatively correlated with reticulocyte. A significant reduction in hepcidin levels is observed after treatment with EPIAO for two weeks. However, this effect

is not observed in iron sucrose alone or combined with EPIAO treatment. Intravenous iron sucrose is an effective agent for the maintenance iron therapy.

The positive correlation between serum hepcidin and iron parameters may reflect the known regulation of hepcidin of the iron metabolism. This finding was supported by other studies [11-13]. However, inconsistent results remain exist. There was no significant correlation between ferritin and hepcidin level in a more recent publication [14]. There are many factors involved in hepcidin production, including artificial fluid retention, infection or inflammation. Inflammation and iron overload has been shown to increase hepcidin synthesis, whereas increased erythropoiesis suppresses hepcidin production [15, 16]. However, there were no significant differences on hs-CRP levels between MHD patients and healthy controls, which suggesting inflammation appeared to be not prominent in our patients. Therefore, inflammation may be not a confounding factor in the current study.

Insufficient production of erythropoietin and iron deficiency is the main cause of anemia among MHD patients. An optimal iron status is critical to achieve maximum benefits from ESAs. Absolute or functional iron deficiency could reduce benefits from ESAs. Hepcidin interfers iron recycling by blocking iron absorption from the duodenum and iron release from the liver or macrophages [17]. Hepcidin could serve as an indicator of functional iron deficiency in MHD patients [18]. In this study, increase in EPIAO doses in epoetin-treated patients resulted in a significantly decline in serum hepcidin level and rise of serum erythropoiesis concentration. Elevated serum erythropoiesis could suppress hepcidin production. While intravenous iron sucrose alone or in combination with EPIAO did not obviously affect the serum hepcidin level. Higher serum hepcidin level in intravenous iron sucrose-treated patients might be correlated with increasing iron loading or decreasing availability of iron for low serum erythropoiesis level. These findings indicated that removal of serum hepcidin level and blockage of external cause of hepcidin production may reduce the dose of ESAs and improve the iron absorption and utilization.

There are several limitations in this study. First, the sample size of participants was relatively small, which may have a lower statistical power. Second, this study was not a randomized controlled trial, patients selection bias cannot be excluded. Finally, decline in glomerular filtration rate affected the serum hepcidin level and the difference in glomerular filtration rate might have influenced our observed results.

In conclusion, the current study shows that serum hepcidin level in MHD patients is positively correlated with serum iron, serum ferritin, or transferrin saturation, and negatively correlated with reticulocyte in the MHD patients. EPIAO alone but not intravenous iron sucrose treatment is associated with a decline in serum hepcidin level. Reduction of serum hepcidin level and sufficient ESAs supplementation may improve erythropoiesis and restore iron homeostasis.

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Disclosure of conflict of interest

None.

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