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Hepcidin and iron metabolism disorders in patients with chronic kidney disease

Hepcidin i poremećaji metabolizma gvožđa kod bolesnika sa hroničnom bubrežnom bolešću

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Abstract

Bacground/Aim. Hepcidin may play a pathogenetic role in iron metobolism disorders. The aim of this study was to determine the correlation between hepcidin concentration and parameters of iron metabolism in patients with different stage of chronic kidney disease (CKD). **Methods.** The study involved 104 patients with CKD: 64 on hemodialysis (HD) and 40 patients in pre-dialysis stadium (pre-HD) with adequate erythropoetin therapy and iron supplementation. The HD group was divided in four subgroups according to the level of serum ferritin (up to 100; 100–199; 200–499 and over 500 ng/mL). Parameters of anemia, iron status, inflamation and hepcidin level were evaluated. **Results.** The HD patients had a significantly lower eritrocyte count, erythrocytes indexes, hemoglobin and transferrin saturation and significantly higher iron, ferritin, hepcidin and total iron binding capacity (TIBC). The HD subgroups up to 199 ng/mL of serum feritin had lower high-sensitivity C-reactive protein (hsCRP), iron and higher unbuffered iron binding capacity (UIBC), transferrin saturation and TIBC compared to the HD subgroups over 200 ng/mL. The lowest and the highest ferritin subgroups had the highest hepcidin level and it showed significant correlation with ferritin. **Conclusion.** Hepcidin may serve as a marker for better diagnosing and monitoring anemia and iron metabolism disorders in CKD.

Key words:
iron; ferritins; anemia; kidney failure, chronic.

Apstrakt

Uvod/Cilj. Hepcidin može imati patogenetsku ulogu u poremećajima metabolizma gvožđa. Cilj ovog istraživanja bio je da se utvrdi povezanost koncentracije hepcidina i parametara metabolizma gvožđa kod bolesnika u različitim fazama hroničnog bubrežnog oboljenja (CKD). **Metode.** Studija je obuhvatila 104 bolesnika sa CKD: 64 na hemodijalizi (HD) i 40 bolesnika u zadnjoj fazi bubrežne bolesti u predijaliznom stadijumu sa adekvatnom eritropoetinskom terapijom i suplementima gvožđa. Grupa HD bila je podeljenja u četiri podgrupe prema nivou serumskog feritina (do 100; 100–199; 200–499 i preko 500 ng/mL). Određivani su parametri anemije, statusa gvožđa, inflamacije i hepcidina. **Rezultati.** Bolesnici HD grupe imali su znatno niži broj eritrocita, eritrocitne indekse, hemaglobin i saturaciju transferina i znatno veće vrednosti gvožđa, feritina, hepcidina i totalni kapacitet vezivnog gvožđa (TIBC). HD podgrupe sa vrednostima feritina do 199 ng/mL imale su niži visokosenzitivni C-reaktivni protein (hsCRP) i nivo gvožđa i visok slobodni kapacitet vezivanja gvožđa (UIBC) u odnosu na HD podgrupe za preko 200 ng/mL feritina u serumu. Podgrupe sa najvišim i najnižim vrednostima feritina imale su najveće vrednosti hepcidina što je bilo u značajnoj korelaciji sa vrednostima feritina. **Zaključak.** Hepcidin može poslužiti kao marker za bolju dijagnozu i praćenje anemije i poremećaje metabolizma gvožđa u CKD.

Ključne reči:
gvožđe; feritin; anemija; bubreg, hronična insuficijencija.

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Introduction

Anemia is a major complication of chronic uremia in the pre-dialysis period and during maintenance dialysis. Anemia develops from the moderate stage of chronic kidney disease (CKD), worsens with the progression of renal failure and is not, or is only incompletely, improved by maintenance dialysis [1, 2].

Iron deficiency can occur in all hemodialysis patients as a result of continuing blood losses and increased iron utilization as a result of erythropoiesis-stimulating protein therapy [3].

Hepcidin is a systemic key regulator of iron homeostasis found on the surface of macrofages and enterocyte that induces internalization and degradation of ferroportin [4, 5]. Thus, hepcidin inhibits the release of iron from macrofages reducing the iron absorption in the bowels. In addition, hepcidin may directly prevent proliferation and erythroid-progenitor survival (synthesis) [6]. Increased iron stores and inflammation induce hepcidin production, whereas hypoxia, anemia, iron deficiency, increased erythropoiesis and recombinant human erythropoietin (rHuEPO) attenuate hepcidin synthesis [7–11].

Hepcidin may play a pathogenetic role in iron metabolism disorders, as well as rHuEPO resistance. However, the molecular hypoxic or anemic regulation mechanisms are still unclear. Several studies have shown that erythropoesis induction is sufficient to reduce hepcidin synthesis, and not hypoxia or anemia [9, 12–14]. The erythropoiesis is increased by rHuEPO, and iron should be mobilized from the storages in order to meet the demands of the bone marrow. A significant reduction in circulating hepcidin level caused by rHuEPO therapy may explain the increased iron release. The connection between hepcidin synthesis and erythropoiesis points to the erythrocytes and liver regulator existence [7, 9, 15].

The aim of this study was to determine the correlation between hepcidin concentration and parameters of iron metabolism in patients with different degree of CKD.

Methods

The study was performed at the Clinic of Nephrology, Clinical Center Niš and Clinical-Biochemical Laboratory of the Military Hospital in Niš. A complete patient history was noted for all the investigated patients. The study involved 104 pateints with CKD divided into two groups: the hemodialysis (HD) group and pre-dialysis stadium group (pre-HD) comprised 64 patients who were dialyzed three times per week for 4 hours *via* polysulfone dialyzers (F6 and F7 HPS Fresenius Medical Care, Bad Homburg, Germany), using the bicarbonate dialysis solutions and standard heparinization. All the HD patients were on rHuEPO and oral iron therapy [European Best Practice Guidelines (EBPG)] and if they had absolute (ferritin < 100 ng/mL) or functional [ferritin >100 ng/mL, transferrin saturation (TSAT) < 20%] iron deficiency [16], we initiated the IV Venofer (Lek Ljubljana) (iron sucrose) protocol[17]. Pre-HD stadium was defined as 3 [glomerular filtration rate (GRF) 30–59 mL/min/1.73m^2] and 4 (GFR 15–29 mL/min/1.73m^2) stadium of CKD by the National Kidney Foundation[17]. Pre-HD group consisted of 40 patients who were in the stadium with adequate erythropoetin therapy and iron oral supplementation. According to the EBPG for studying anemia in patients with CKD, iron deficiency is described as the main cause of erythropoiesis stimulating agents treatment resistance, whether there is absolute (ferritin < 100 ng/mL, transferrin saturation < 20%) or functional (ferritin > 100 ng/mL and transferrin saturation < 20%) iron deficiency. That is why the HD group was divided in four subgroups according to the level of serum ferritin (ferritin concentration up to 100 ng/mL; from 100–199 ng/mL; from 200–499 ng/mL and over 500 ng/mL).

The exclusion criteria were: less than 18 years old, evidence of acute infection or trauma in the last four weeks, history of parenteral iron injection in the last 14 days, history of blood transfusion in the last one month, hemoglobinopathy, malignancy, recent overt blood loss, and post-transplant status. All the patients showed no signs of infection or hepatitis B and C.

Blood was extracted using the closed vacuum system for all the patients. Tubes with EDTA anticoagulant were used for the hematological parameters, whereas for the biochemical parameters, the tubes were without anticoagulant. After sampling, blood was put into a centrifuge and separated from the serums out of which the following biochemical and hematological parameters were evaluated: the overall blood count red blood cells (RBC); hemoglobin (Hb); hematocrit (HCT); median cell volume (MCV); median concentration of hemoglobin (MCH); median cell hemoglobin concentration (MCHC) were determined on hematological autoanalyzer ADVIA 120 Simens ex Bayer. Iron, total iron binding capacity (TIBC), unbuffered iron binding capacity (UIBC), transferrin saturation, albumin and high-sensitivity C-reactive protein (hsCRP) were determined on a biochemical analyzer (Dimension, Dade Behring), while ferritin was assayed by using a commercially available immunohistochemical test (Cobas e 411 Rosch).

Hepcidin was determined using the commercial ELISA test (DRG, Marburg, Germany). The measure range of the assay is 0.9–140 ng/mL. The analytical low level of sensitivity of the DRG ELISA was calculated by subtracting 2 standard deviations from the mean of 20 replicate analyses of the Zero Standard (SO) and was found to be 0.9 ng/mL.

The research was approved by institutional review boards of Faculty of Medicine, University in Niš and institutional Ethics Committee's number 01-4097-1/06.07.2011. Inform consent was obtained from all the participants.

Statistical analysis was performed using the standard descriptive methods (mean ± SD), and corresponding analytical tests. Levene's Test for Equality of Variances was performed to determine the equality of variances, and appropriate independent samples, while the Student's *t*-test was used to compare the means. The intergroup variability was determined using the ANOVA test and *post hoc* analysis, and the Mann-Whitney test was used as a non-parametric test. The correlation between the results was tested with the Pearson´s Correlation Coefficient.

Results

In the HD group, 42 male and 22 female patients were analyzed unlike the pre-HD group, in which 34 male and 6 female patients were analyzed. Clinical characteristics and parameters of anemia of investigated groups with CKD are shown in Table 1. Baseline characteristics of CKD patients did not show statistically significant difference between the HD and pre-HD group, but the patients on hemodialysis had a significantly lower number of RBC, Hb concentration and HCT, MCV, MCH values ($p < 0.01$) and transferrin saturation ($p < 0.05$) compared to the pre-HD group. Higher iron concentrations and TIBC ($p < 0.05$) were found in the HD patients group (Table 1).

Patient division on the basis of ferritin levels in the patients with CKD on hemodialysis is shown in Tables 2 and 3.

Hematological anemia parameters did not show any significant differences in the subgroups of HD group patients with various ferritin value intervals (Table 2).

The ANOVA analysis showed the existence of significant intergroup differences in iron, transferrin saturation, UIBC, TIBC, hsCRP and hepcidin values among the tested patients groups. *Post hoc* analysis revealed that the patients with ferritin levels < 100 ng/mL and 100–199 ng/mL had significantly lower hsCRP as well as significantly higher UIBC, and transferrin saturation levels compared to the groups with ferritin 200–499 ng/mL and > 500 ng/mL. The patients with ferritin levels < 100 ng/mL and 100–199 ng/mL had significantly lower iron and significantly higher TIBC levels compared to the group with ferritin > 500 ng/mL. The HD patients group with lowest < 100 and highest > 500

Table 1

The clinical characteristics and parameters of anemia in the patients with chronic kidney disease (CKD)

Parameters	Groups of patients	
	HD (n= 64)	pre-HD (n = 40)
Age (years), $\bar{x} \pm SD$	62.6 ± 6	65.1 ± 4.7
CKD history (years), $\bar{x} \pm SD$	5.78 ± 4	8 ± 4.7
Hemodialysis history (years), $\bar{x} \pm SD$	6.97 ± 6.15	–
GRF (mL/min/1.73m^2), $\bar{x} \pm SD$	7.85 ± 4.2	36.7 ± 3.8
Hypertension n, (%)	56 (88)	23 (61)
Smoking, n (%)	0/0	3/16
Systolic TA (mmHg), $\bar{x} \pm SD$	130 ± 7.9	132 ± 13.1
Diastolic TA (mmHg), $\bar{x} \pm SD$	80.3 ± 7.8	82.5 ± 8
RBC (T/L), $\bar{x} \pm SD$	3.08 ± 0.59	3.79 ± 0.20**
Hb (g/L), $\bar{x} \pm SD$	97.7 ± 20.19	112.15 ± 3.78**
HCT	29.57 ± 5.87	33.67 ± 1.21**
MCV	90.54 ± 2.03	94.58 ± 4.61**
MCH	30.28 ± 0.73	31.55 ± 1.75**
MCHC	330.8 ± 5.73	332.08 ± 15.77
Fe (µmol/L), $\bar{x} \pm SD$	20.56 ± 7.18	16.98 ± 2.03*
Transferrin saturation (%),$\bar{x} \pm SD$	19.34 ± 11.06	24.6 ± 4.8*
UIBC (µmol/L), $\bar{x} \pm SD$	39.64 ± 8.96	40.79 ± 5.68
TIBC (µmol/L), $\bar{x} \pm SD$	53.14 ± 21.92	41.03 ± 7.22*

*** $p < 0.05$; ** $p < 0.01$ *vs* hemodialysis (HD); RBC – red blood cells; Hb – hemoglobin; HCT – hematocrit; MCV – median cell volume; MCH – median concentration of hemoglobin; MCHC – median cell hemoglobin concentration; UIBC – unbuffered iron binding capacity, TIBC – total iron binding capacity; GRF – glomerular filtration rate**

Ferritin (Figure 1) and hepcidin (Figure 2) concentration were significantly higher ($p < 0.01$) in the HD group compared to the pre-HD group.

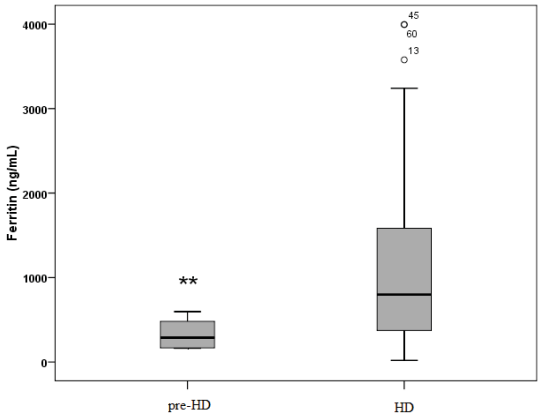

Fig. 1 – Ferritin concentration in the examined groups.

****$p < 0.01$ vs hemodialysis (HD), Mann-Whitney test; Boxplot summarizes the median, quartiles (25–75. percentiles), extreme values and outliers (o), error bars represents 95% confidence intervals (CI)**

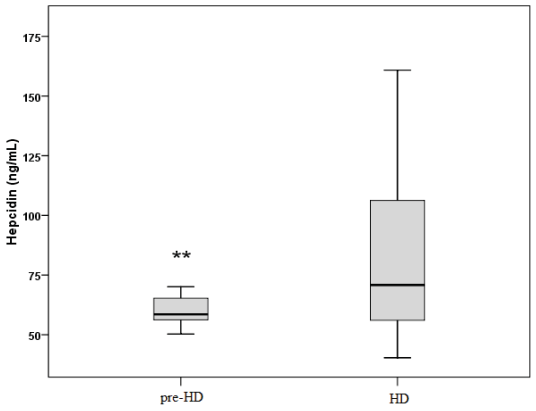

Fig. 2 – Hepcidin concentration in the examined groups

****$p < 0.01$ vs hemodialysis (HD); Mann-Whitney test; Boxplot summarizes the median, quartiles (25–75. percentiles) and extreme values, error bars represents 95% confidence intervals (CI)**

Table 2

The parameters of anemia according to the level of ferritin in the hemodialysis patients

Parameters	Ferritin (ng/mL)			
	< 100 (n = 12)	100–199 (n=12)	200–499 (n = 14)	> 500 (n = 26)
RBC (T/L)	3.32 ± 0.72	2.80 ± 0.53	3.28 ± 0.45	3.0 ± 0.58
Hb (g/L)	98.67 ± 26.99	89.42 ± 14.13	106.36 ± 17.37	96.42 ± 19.62
HCT (%)	30.69 ± 7.07	26.98 ± 4.29	31.68 ± 5.15	29.13 ± 6.05
MCV (fL)	92.63 ± 4.02	94.52 ± 4.08	94.89 ± 5.93	95.37 ± 4.28
MCH (pg/cell)	30.73 ± 1.85	31.58 ± 1.46	31.66 ± 2.15	31.86 ± 1.59
MCHC (g/dL)	323.0 ± 30.63	335.25 ± 8.15	332.86 ± 9.39	334.38 ± 9.45

The data are presented as mean ± SD; n – number of patients; RBC – red blood cells; Hb – hemoglobin; HCT – hematocrit; MCV – median cell volume; MCH – median concentration of hemoglobin; MCHC – median cell hemoglobin concentration

Table 3

Iron (Fe) status according to the level of ferritin in the hemodialysis patients

Variables	Ferritin (ng/mL)			
	< 100 (n = 12)	100–199 (n = 12)	200–499 (n = 14)	> 500 (n = 26)
Fe (μmol/L)	16.38 ± 3.26 [a]	16.54 ± 6.73 [a]	22.65 ± 6.97	23.23 ± 7.38
Transferin saturation (%)	29.38 ± 6.30 [b]	27.83 ± 7.45 [b]	16.56 ± 8.53	12.29 ± 9.47
UIBC (μmol/L)	48.42 ± 7.78 [b]	47.37 ± 8.38 [b]	39.16 ± 7.39	32.07 ± 8.25
TIBC (μmol/L)	58.10 ± 21.74 [a]	55.82 ± 18.74 [a]	46.90 ± 13.3	36.83 ± 6.94
Hepcidin (ng/mL)	92.51 ± 40.99 [c]	61.34 ± 12.97	65.19 ± 25.48	96.27 ± 29.1 [c]
hsCRP (mg/L)	3.73 ± 2.26 [b]	3.82 ± 1.90 [b]	6.02 ± 3.14	7.95 ± 2.11
Albumin (g/L)	20.26 ± 3.89[d]	30.74 ± 3.45	34.97 ± 3	27.15 ± 4.56

The data are presented as means ± SD; *Post hoc* Tukey HSD test: [a] $p < 0.05$ vs. >500; [b] $p < 0.05$ vs 200–499 and > 500; [c] $p < 0.05$ vs 100–199 and 200–499; [d] $p < 0.05$ vs all the rest; TIBC – total iron binding capacity; UIBC – unbuffered iron binding capacity; hsCRP – high-sensitivity C-reactive protein

ng/mL ferritin values had significantly higher hepcidin compared to 100–199 ng/mL and 200–499 ng/mL ferritin subgroups. The patients with ferritin levels < 100 ng/mL showed statistically significant lower albumin levels compared to the other groups of patients (Table 3).

Hepcidin showed a significant correlation with ferritin in both patient groups (HD – $r = 0.46$, $p < 0.01$; pre-HD – $r = 0.69$, $p < 0.01$), while hsCRP was in a significant correlation with hepcidin in HD patients only ($r = 0.565$, $p < 0.05$). In the HD patients albumin was significantly negatively correlated with hepcidin ($r = -0.487$, $p < 0.05$). In HD patients with chronic renal failure, bivariate analysis showed no significant correlation of hepcidin with any parameters of anemia. In pre-HD patients with chronic renal failure, hepcidin correlated inversely with RBC ($r = -0.81$, $p < 0.01$), MCV ($r = -0.738$, $p < 0.01$) and MCH ($r = -0.535$, $p < 0.05$) (Table 4).

Discussion

Determination of iron deficiency level in patients on hemodialysis is much more difficult than in normal population. In connection with the homeostasis of ferritin, three types of anemia have been identified in patients on hemodialysis (absolute, functional deficiency and reticuloendothelial blockade) even if there are still doubts in official markers and indicators that are currently used for identification [18].

Table 4

The correlation of hepcidin with iron (Fe) parameters in the hemodialysis (HD) patients

Parameters	Hepcidin	
	HD group	pre-HD group
Ferritin	0.467**	0.694**
hsCRP	0.565*	0.285
Albumin	-0.487*	0.015
% sat	0.156	-0.172
TIBC	-0.187	-0.165
UIBC	-0.181	0.012
Fe	0.062	-0.169
RBC	0.026	-0.811**
Hb	-0.063	0.317
HCT	0.015	0.254
MCV	0.085	-0.738**
MCH	-0.005	-0.535*
MCHC	-0.257	-0.216

*** – significant correlation at $p < 0.05$; ** – significant correlation at $p < 0.01$**
TIBC-total iron binding capacity; UIBC – unbuffered iron binding capacity; RBC –red blood cells; Hb – hemoglobin; HCT – hematocrit; MCV – median cell volume; MCH – median concentration of hemoglobin; MCHC – median cell hemoglobin concentration; hsCRP – high-sensitivity C-reactive protein

A routine monitoring of ferritin status in patients on hemodialysis is of vital importance in order to prevent the occurrence of iron deficiency and to avoid constantly increased value in assessing ferritin status. Insufficient iron supplies may lead to anemia as a result of iron defficiency [17], which in turn causes changes in the functioning of cardiovascular system (left ventricle hypertrophy, reduced ventricular hypertrophy ejection fraction and congestive heart disease), exhaustion and reduced quality of life [19–21]. Contrary to the above mentioned the correction of anemia leads to the improvement of heart morphology, reduction of the length of stay in hospital and improves the quality of life [22–23].

Well-known hematological parameters of anemic syndrome RBC, Hb, HCT, MCV and MCH are reduced in patients with hyperbaric oxigenation and in those on hemodialysis. However, in the HD dialysis group of patients increased iron, ferritin and hepcidin levels were observed, while transferrin saturation was significantly decreased. These data are consistent with a recent examination of De Dominico et al. [24] who confirmed the presence of inhibitory effects of hepcidin on iron levels. This relationship is explained by the mechanism of negative feedback because ferroportin loss from the surface of the cells causes a reduction of ferritin in plasma, which creates low transferrin saturation. In this way, less iron is transported to erythroblasts, leading to chronic anemia, which interferes with the production of hepcidin. On the other hand, iron is, trapped inside macrophages and ferritin [9].

A significant positive correlation between RBC number and hepcidin level was found in patients on pre-dialysis stage. This indicates the importance of monitoring hepcidin in patients on dialysis during the correction of anemic syndromes and disorders of iron metabolism, since the increased levels of transferrin and better fulfillment of erythrocytes does not reflect on an increase in their number. This may be a consequence of the proinflammatory state in the patients on hemodialysis. Inflammation can be caused by the dialysis itself, which leads to the increased concentrations of circulating cytokines such as interleukin-1 (IL-1) and IL-6, alpha-tumor necrosis factor (TNF-α) or γ-interferon [25–30] and hepcidin. They can directly affect the biological function of erythropoietin, which in turn causes the retention of iron in macrophages / monocytes, accompanied by reduced erythropoiesis of iron [15, 31].

Hepcidin synthesis is increased in iron overload conditions and during inflammation, while the decreased synthesis may be due to iron deficiency and anemia [9]. This is indicated by the positive correlation of hepcidin and ferritin in both groups, and hepcidin and hsCRP in patients with HD. Statistically higher levels of hsCRP were pointed to an inflammatory state in the HD group of patients and in the subgroups of patients with ferritin 200–400 ng/mL and the group with ferritin > 500 ng/mL in our study, which coincides with the findings of Ashby et al. [32]. This phenomenon can be explained by previous studies in cultures of human hepatocytes in which hepcidin is induced by IL-6 but not IL-1 or TNF-α [33]. Three different modes of regulation of hepcidin have been noticed: inflammatory, which depends on IL-6, regulation of iron levels (mainly determined by transferrin saturation) and suppression of hepcidin synthesis caused by hypoxia and anemia. It is believed that frequent use of iron may reduce the stimulation of hepcidin by creating a reduction in saturation transferin [34–36]. Pro-inflammatory state on the other hand can cause erythropoietin resistance [37]. Significantly higher levels of hepcidin in the group with ferritin levels > 500 ng/mL can be expected due to excessive amounts of iron, where the increased synthesis of hepcidin causes a negative feedback mechanism.

However, proinflammatory condition is not found in the group of patients with ferritin < 100 ng/mL, and there were statistically significantly higher levels of hepcidin. Judging by the significantly lower levels of albumin and negative correlation with hepcidin in these groups of patients, the reason is to be sought in the disorder of liver synthesis function. The research of Detivaud et al. [38] found a direct correlation of liver function with the level of hepcidin, while the research Małyszko et al. [39] showed a direct negative correlation of albumin and hepcidin. On the other hand, specific circulating binding proteins hepcidin are the α-2 macroglobulin and albumin [40], all of which can explain the increased levels of hepcidin in these patients.

Ferritin and transferrin saturation are irreplaceable markers in determining the iron status, which hepcidin is not comparable with. Hepcidin together with ferritin and transferrin saturation can give more insight in the evaluation of iron status in patients with chronic kidney failure and hemodialysis. No connection of hepcidin and transferrin saturation was found in this paper, but there was a direct correlation between hepcidin and ferritin. In addition, in the ferritin groups from 100 ng/mL to 499 ng/mL in the HD patients, a slight decrease in hepcidin was recorded. We think that it would be most appropriate to determine hepcidin in patients with chronic kidney failure and hemodialysis with the highest ferritin values. Since all the patients were on erythropoietin therapy, which leads to iron overload, the increased hepcidin values could indicate the appearance of erythropoietin resistance. This opinion requires further investigation. Hepcidin is certainly not the marker, at least for the time being, that would be used in clinical practice.

Conclusion

Hepcidin may have an important role as a marker in diagnosing and monitoring the iron metabolism disorders in CKD. It showed maximal values in the lowest and highest ferritin level group and was in linear correlation with ferritin in both patient groups. In this way, the determination of hepcidin may be of clinical importance in better anemia monitoring in both group of patients – pre-HD and HD.

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