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Original Article

Hepcidin and iron status in chronic kidney disease

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Abstract

Hepcidin is a critical inhibitor of iron export from macrophages, enterocytes, and hepatocytes. Given that it is filtered and degraded by the kidney, its elevated levels in renal failure have been suggested to play a role in the disordered iron metabolism of uremia. It is a small defensin-like peptide whose production by hepatocytes is modulated in response to anemia, hypoxia, or inflammation. Hepcidin could also act as an indicator of functional iron deficiency (FID) in chronic kidney disease (CKD) patients. This study was performed to assess hepcidin and its correlations with renal function, iron status parameters {serum iron, serum ferritin, transferrin saturation (TSAT) and soluble transferrin receptor (sTfR)}, inflammatory cytokines (IL-6&IFN-β) and inflammatory markers (CRP) in patients with CKD either on conservative treatment or on maintenance hemodialysis (HD). Serum prohepcidin was higher in HD patients compared to controls and CKD patients. In the whole patient group, serum hepcidin correlated significantly with hemoglobin (Hb), IL-6, creatinine, CRP, sTfR and urinary hepcidin. In HD group prohepcidin correlated significantly with creatinine. Multiple regression analysis showed that prohepcidin was most predicted by serum creatinine and CRP. Elevated prohepcidin levels in HD patients studied could mainly be due to its accumulation in impaired renal function in addition to low-grade inflammation, frequently encountered in this population.

Introduction

Anemia is a cardinal feature of CKD and has a substantial impact on morbidity and mortality. It is mainly due to erythropoietin deficiency (Epo) [1]. This is often associated with iron deficiency (ID) [2,3]. Iron metabolism is also impaired in CKD and frequently may complicate the management of anemia. Functional iron deficiency (FID) is characterized by the presence of adequate iron stores but with an inability to sufficiently mobilize iron for adequate support of erythropoiesis. FID frequently exists among CKD patients with renal failure [4]. Anemia of chronic disease (ACD), also known as anemia of inflammation, occurs with infections, cancer, autoimmune disease and CKD [5]. ACD can present with or without ID and its treatment will depend on its cause [6,7].

Hepcidin is small cysteine-rich polypeptide produced by the liver from an 84 amino acid precursor (pro-hepcidin) [8]. It was discovered in 2000, and appears to be the master regulator of iron homeostasis in humans and other mammals [9,10]. Inflammation causes an increase of production of hepcidin, which is a potent mediator of ACD [1]. It is partially responsible for iron sequestration seen in ACD [11]. Hepcidin production in patients with CKD depends on iron status, inflammation, anemia, hypoxia and EPO [5], and hepcidin also acts as an acute-phase reactant induced by inflammation [9,12].

Prohepcidin levels in CKD were increased and correlated negatively with the glomerular filtration rate [5]. The difference in serum prohepcidin and urinary hepcidin did not reach statistical significance; however, there was a tendency toward higher values of both prohepcidin and urinary hepcidin in erythropoietin hypo-responsive patients. This hypo-responsiveness to erythropoietin therapy occurring in dialyzed patients is mainly associated with sub clinical inflammation than with hepcidin excess [13].

Proinflammatory cytokine interleukin-6 (IL-6) was used to see if it resulted in increased hepcidin levels. After infusion of IL-6 in human volunteers, hepcidin levels increased while iron saturation decreased. This showed that IL-6 was sufficient to up-regulate hepcidin [14].

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At the molecular level, hepcidin binds to the sole known cellular iron efflux channel, ferroportin, and induces its internalization and lysosomal degradation. Ferroportin is present on enterocytes and macrophages. By inhibiting ferroportin, hepcidin prevents enterocytes of the intestines from secreting iron into the hepatic portal system, thereby functionally reducing iron absorption. Iron release from macrophages is also prevented by ferroportin inhibition. This eventually leads to anemia by decreasing iron availability for erythropoiesis. Conversely, the absence of hepcidin leads to unregulated duodenal iron absorption and subsequent iron overload [15]. In addition to these effects on body iron distribution, hepcidin might also directly inhibit erythroid-progenitor proliferation and survival [8,16].

Erythropoiesis stimulating agents (ESAs) increase the erythropoietic activity, and thus iron must be rapidly mobilized from the stores to satisfy the needs of the bone marrow. The reported decrease in circulating hepcidin levels by ESA treatment may explain the fast iron release. The relationship between hepcidin production and erythropoiesis suggests the presence of a regulator between the erythron and the liver, and several candidates for this role have been proposed, for example the sTfR. Several studies demonstrated that the induction of erythropoiesis and not hypoxia or anemia itself down-regulates hepcidin [16].

A highly accurate laboratory test detecting a biomarker that is not affected by inflammation could be a more reliable method of assessing the body's iron status in the presence of ACD. The sTfR has been introduced as a promising new diagnostic tool for differentiating between IDA and ACD [17]. The circulating sTfR concentration is proportional to cellular expression of the membrane-associated TfR and increases with increased cellular iron needs and cellular proliferation. Furthermore, because serum ferritin reflects the storage iron compartment and sTfR reflects the functional iron compartment, the sTfR/ferritin ratio, based on these two values, has been suggested as a good estimate of body iron [5,18]. In FID, as often occurs in (low grade) inflammatory diseases such as CKD, hepcidin levels will be elevated and the release of iron from the reticulo-endothelial cells is inadequate for erythropoiesis. The currently used parameters of iron metabolism, such as TSAT and ferritin, did not predict the available iron stores [19].

The aim of this study was to assess prohepcidin and its correlations with renal function, iron status, inflammatory cytokines (IL-6 & IFN-β) and inflammatory markers (CRP) in patients with CKD either on conservative treatment or on maintenance HD. As hepcidin has the potential to become a target of treatment, its potential relevance for anemia management in these patients was emphasized.

Material and methods

Study population: This study was conducted on 40 subjects from Nephrology and Dialysis Unit (Theodor Bilharz Research Institute). They were divided according to data from initial clinical and routine laboratory evaluation into the following three groups:

Group A: This included 15 ESRD patients (9 males and 6 females with ages ranging between 33-65 years with a mean of 52±9.9) on regular HD treatment (3 sessions weekly: 4 hours each for a period more than 3months) using Fresenius 4008 B machine, Hemophane filters with 1.4 surface area and sodium acetate solution as a dialyzate.

Group B: This included 15 CKD patients on conservative treatment (8 males and 7females, ages ranging between 19-70 years with a mean of 44±14.9).

None of the patients received blood or blood components transfusion therapy in the past 21 days and none of them was receiving EPO therapy.

The etiology of CKD was variable between the 2 studied patient groups (hypertension, diabetes mellitus, urologic and unknown causes).

Informed consents were obtained in accordance with the Declaration of Helsinki.

Group C: This included 10 (age and sex matched) healthy control subjects, selected from medical and paramedical staff.

Sampling: Blood samples were collected in EDTA anticoagulated vacuum tubes to perform complete blood count (CBC). Blood samples were also collected into iron free vacutainer tubes, allowed to clot and serum was separated as soon as the clot was formed. Serum was collected and distributed into small aliquots for biochemical tests, evaluation of iron profile and measurement of prohepcidin, sTfR, IL6, IFN-β and CRP. Serum samples were stored frozen at –80°C.

Samples were collected from all patients in a fixed time of the day (in the morning) to avoid diurnal variation.

Urine samples (second morning samples) were collected into clean tubes for cyto-pathologic diagnosis and immuno-cytochemical techniques.

Laboratory assays:

- *Routine laboratory investigations:* CBC including Hb concentration, MCV, MCH, MCHC and RDW was performed using hematology analyzer Celtac-MEK 8118 (Nihon Kohden, Japan). Kidney function (blood urea & serum creatinine) and serum albumin were assessed by the conventional methods.
- *Special laboratory investigations:*
  - Iron profile (serum iron, serum ferritin and transferrin) was measured by ELISA technique using commercially available kits (Bayer Diagnostics, U.K.). TSAT was calculated as {serum iron (µg/dl) x 70.9/ serum transferrin (mg/dl)}.
  - sTfR level was measured by ELISA using Quantikine human sTfR kit (R&D systems Inc, USA). Calculation of the ratio sTfR/ log ferritin (sTfR-F Index) was used as a determinant of body iron stores.

- Serum prohepcidin hormone level was measured by using DRG hepcidin prohormone ELISA kit (DRG, Germany).
- IL-6 values were evaluated by ELISA using Quantikine IL-6 (Quantikine IVD, minipolos, USA) test kit.
- Serum values of IFN- β were measured by using PBL Interferon-β ELISA kit (Pestka Biomedical Laboratories, New York, USA).
- Serum CRP levels measured using immunoturbidimetric assay (Randox Laboratories, Ltd, UK). Kone Progress/ specific.
- A urinary hepcidin assay was used to compare values of serum prohepcidin and urinary hepcidin level especially in CKD patients with residual renal function.

- *Cytopathologic processing of urine samples:*
  The collected urine was centrifuged at a rate of 1200–1500 rpm for 15 minutes using Shandon Cytospin (Thermo Fisher Scientific, Waltham, Massachusetts). The sediment was smeared on slides that were pretreated with 3-APTES (3-amino-propyl-triethoxy saline, Sigma-Aldrich Ireland Ltd, Dublin, Ireland). Slides were fixed immediately in 95% ethanol for 24 hours and then stained with hematoxylin and eosin for cytopathologic diagnosis and Papanicolaou's stain [20].
  Immunocytochemical procedures for hepcidin C protein on urine smears were done:
  The slides were treated with 0.3% hydrogen peroxide to inhibit the activity of endogenous peroxidase. Sections were incubated overnight at 4°C with the primary anti-hepcidin antibody (Santa Cruz Biotechnology, Santa Cruz, California). Both antibodies were diluted 1:50 in phosphate buffer saline (PBS) and incubated for 24 hours at 4°C. The following day, the slides were washed 3 times in PBS, and then sections were incubated for 15 minutes with biotinylated secondary antibody and then with avidin-biotin complex horseradish peroxidase solution (Vector, Burlingame, California). After incubation for 10 minutes, the peroxidase activity was revealed by the addition of freshly prepared diaminobenzidine (0.03%) for 20 minutes at 37°C in dark then washed 3 times in PBS or tries-HCL buffer pH 7.6. PBMNC slides and urine slides were counter stained with May-Grunwal-Giemsa stain and Meyer's hematoxylin, respectively.
  Negative controls were processed in an identical manner by substitution of primary antibody with a normal rabbit IgG.
- *Assessment of immunocytochemical staining:*
  Using a standard light microscope, all slides (specimens) were categorized as either positive or negative in terms of staining for the hepcidin C. Those specimens with cytoplasmic positive brownish staining were considered positive for hepcidin C protein, slides were classified as negative if the staining level was comparable with that of the negative control slides. Cells were counted at 400 magnifications. An average of 100 cells within 10 fields was counted/section. Two pathologists reviewed all slides independently (figure 1&2).

Statistical analysis

All statistical analyses were performed using the SPSS for Windows, version 11 (software). Results were expressed as means ± standard deviation. Comparing means was performed by one-way ANOVA Post HOC LSD test. To evaluate correlations among the variables, a Pearson correlation test or Spearman correlation test was used as appropriate. A $p < 0.05$ was considered statistically significant. The Mann-Whitney U test was used as appropriate. Multivariate regression analysis was used to determine the best predictors of prohepcidin level (used as the dependent variable).

Results:

The results obtained in this study are shown in tables 1&2. The correlation analysis is shown in tables 3-6.

Table1. Results of studied parameters in different patient groups.

Parameters	*Control group*	*CKD*	*HD*
Hb (g/dl)	13.7 ±0.6	9.9±1.2[a]	8.3±0.5 [ab]
MCV (fl)	85.5±3	76.4±2.6[a]	72±2.5[ab]
MCH (pg)	30.4 ± 1.4	24.9 +/- 2.6[a]	22.±1.6 [ab]
MCHC (g/dl)	34.1 ±0.9	31.5 +/- 2.3[a]	29±1.3 [ab]
RDW (%)	11.8 ±0.1	15.6±1.6[a]	16.9±1.3[ab]
sFe (µg/dl)	104± 13.5	55.7±15.7[a]	49.4±15[a]
Ferritin (ng/ml)	111± 33.5	70.1±22.0[a]	61±20.7[a]
TSAT(%)	35.2±1.9	20.2± 3.5[a]	18.9±4.6[a]
sTfR (µg/ml)	1.6±0.3	2.8±0.7[a]	3.4±0.5[ab]
sTfR/log F Index	0.8±0.1	1.4±0.3[a]	1.6±0.3[a]
Prohepcidin (ng/ml)	72.9±11.9	84±18.6	143±25[ab]

IL-6(pg/ml)	11.2±3.1	21.5±5.6	42.7±24[ab]
IFN-β(pg/ml)	125±26.8	252±189	300±223[a]
CRP (mg/l)	1.9±05	5±0.9[a]	8.5±2.3[ab]
sUrea (mg/dl)	23.7±2.7	117.8 ±10.9[a]	121±10.9[a]
sCreatinine (mg/dl)	0.8±0.2	3.9±2.1[a]	8.3±2.6[ab]
sAlbumin (g/dl)	4.1±0.4	2.5±1.6[a]	2.8±1.6[a]

A: Significant difference from control group
b: Significant difference from CKD group
c: Significant difference from HD group (Significant difference: $p<0.05$)

Table 2. Results of urinary hepcidin evaluation:

			Control	*CKD*	*HD*	*Total*
Urinhepc	Neg	Count	7	4		11
		% negative	63.6%	36.4%		100%
		% within gps	70.0%	26.7%		27.5%
		% of Total	17.5%	10.0%		27.5%
	+Positive	Count	3	7		10
		% + positive	30.0%	70.0%		100%
		% within gps	30.0%	46.7%		25%
		% of Total	7.5%	17.5%		25%
	++Positive	Count		4	9	13
		% ++positive		30.8%	69.2%	100%
		% within gps		26.7%	60.0%	32.5
		% of Total		10.0%	22.5%	32.5
	+++Positive	Count			6	6
		% +++positive			100.0%	100%
		% within gps			40.0%	15%
		% of Total			15.0%	15%
Total		Count	10	15		40
		% within URINHEPC	25.0%	37.5%	37.5%	100%
		% within gps	100%	100%	100%	100%
		% of Total	25%	37.5%	37.5%	100%

Mann-Whitney test proved significant difference between both patient groups (CKD & HD) when compared to control group ($p<0.05$ & $p<0.001$ respectively), while a highly significant difference was found between the two groups (HD &CKD) ($p< 0,001$).

Urinary cytology of hepcidin

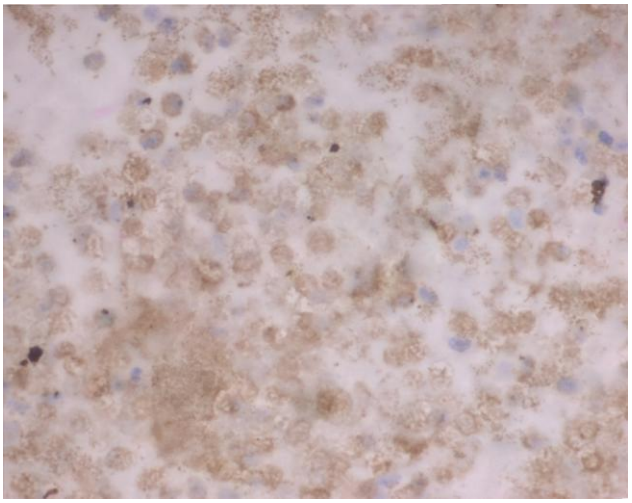

Fig.1. Urine cytology from HD case, marked positivity for hepcidin C as brownish cytoplasmic granules (Immunostain, hepcidin antibody, DAB, X400).

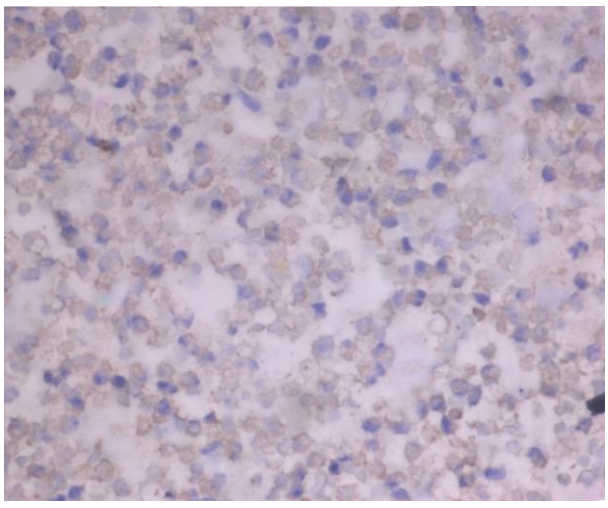

Fig.2. Urine cytology from CKD case, mild positivity for hepcidin C as brownish cytoplasmic granules (Immunostain, hepcidin antibody, DAB, X400).

Table 3. Significant correlations between Hb and other parameters in whole patient group.

Correlations	*Whole patient group*
Hb *vs* Prohepcidin	-0.69**
Hb vs IL-6	-0.50**
Hb *vs* IFN-β	-0.35*
Hb *vs* sTfR	-0.93**
Hb vs sTfR/F ratio	-0.89**
Hb *vs creatinine*	-0.78**
Hb vs CRP	-0.76**
Hb vs sFn	0.78 **
Hb vs sFe	0.87**
Hb vs TSAT	0.897**

** Correlation is significant at the 0.001 level
* Correlation is significant at the 0.05 level

Table 4. Significant correlations between Prohepcidin and other parameters in whole patient group

Correlations	*Whole patient group*
Prohepcidin *vs* Hb	-0.69**
Prohepcidin *vs* IL-6	0.59**
Prohepcidin *vs* sTfR	0.47*
Prohepcidin *vs* RDW	0.48*
Prohepcidin *vs* Urinary Hepcidin	0.60**
Prohepcidin *vs* sCreatinine	0.64**
Prohepcidin *vs* CRP	0.68**
Prohepcidin *vs* MCV	-0.43*
Prohepcidin *vs* MCH	-0.52*

** Correlation is significant at the 0.001 level
* Correlation is significant at the 0.05 level

Table 5. Significant correlations between IL-6 and other parameters in whole patient group

Correlations	*Whole patient group*
IL-6 *vs* Urinary hepcidin	0.52*
IL-6 *vs* sCreatinine	0.46*
IL-6 *vs* CRP	0.57*

*Correlation is significant at the 0.05 level

Table 6. Significant correlations between sTfR and other parameters in whole patient group

Correlations	*Whole patient group*
sTfR *vs* Urinary hepcidin	0.43*
sTfR *vs* RDW	0.88**
sTfR *vs* sCreatinine	0.52*
sTfR *vs*sTfR/F ratio	0.98**
sTfR *vs* sFe	-0.67**
sTfR *vs* TSAT	-0.57*
sTfR *vs* Ferritin	-0.71**

** Correlation is significant at the 0.001 level
* Correlation is significant at the 0.05 level

In CKD patients there were no significant correlations between pro-hepcidin levels, hemoglobin, serum iron, ferritin, TSAT, CRP and albumin. However, urinary hepcidin correlated positively to CRP (R = 0.570, p <0 .001). In HD dependent patients, prohepcidin correlated positively to serum creatinine (R = 0.707, p <0 .05). In the control group, pro-hepcidin level was not related to any of the above studied parameters.

To discriminate the types of anemia in patients studied (True ID vs, ACD with or without ID) sTfR and sTfR/log ferritin ratio were used. sTfR levels were significantly higher in the IDA group compared to ACD group (p<0.001). All cases in IDA group had sTfR/log serum ferritin ratio > 2.55 and all patients with ACD with or without associated iron deficiency had sTfR/log serum ferritin ratio < 2.55(21) (table 7).

Table 7. Results of parameters studied in ID and ACD with or without ID.

		Prohep	*IL6*	*IFN-β*	*sTfR*	*Hb*	*TSAT*	*RDW*	*Ferritin*	*sTfR Ratio*	*CRP*
Index > 2.5 = True IDA	Mean	121.6	33.4	326.0	3.7**	8.0	15.4**	17.5	42.5**	2.6**	6.6
	N	14	14	14	14	14	14	14	14	14	14
	Std. Dev.	23.9	23.6	222.8	.18	.26	2.8	1.2	4.7	.049	1.8
Index <2.5 = ACD with ID	Mean	105.2	29.4	241.3	2.7	9.8	22.2	15.5	81.1	1.4	6.8
	N	18	18	18	18	18	18	18	18	18	18
	Std. Dev.	42.7	17.5	185.6	.52	1.1	1.8	1.3	11.5	.29	2.8

**Significant difference from true IDA group
**(Significant difference: p<0.001)

We found true IDA in 14 patients with renal insufficiency {6 (40% of CKD patients) & 8 (53.3% of patients on HD)} while ACD with or without ID were detected in 18 patients {9 (60% patients with CKD & 7 (46.7% of patients on HD)}. Comparing the variables predicting the presence of ID between the two groups (Table7) revealed that sTfR, sTfR/ log ferritin ratio, TSAT, and ferritin showed a statistically significant difference ($p < 0.001$), while prohepcidin, IL-6, IFN-β and CRP did not show a statistically significant difference.

Multivariate regression analysis was used to determine the best predictors of prohepcidin level (used as the dependent variable). The most predictive parameters for prohepcidin were CRP and serum creatinine ($R^2 = 0.70$).

Discussion

Anemia is common in patients with renal insufficiency. Epo deficiency is by far the major cause, followed by iron deficiency, although shortened erythrocyte survival due to hemolysis, bleeding and oxidative stress may contribute [16,22].

Although hepcidin is the only known biologically active form, we actually measured pro-hepcidin (precursor protein) levels. However, detection and quantification of serum hepcidin still encounters technical difficulties and reliable assays to detect serum hepcidin level are not generally available, in contrast to pro-hepcidin assays that are easier to access. Due to these limitations in hepcidin measurement, studies evaluated serum prohepcidin levels in patients with CKD. Significant changes in prohepcidin concentration have been reported in ferroportin disease and in patients with CKD. Prohepcidin levels were increased and correlated negatively with the glomerular filtration rate [16].

In the present study, prohepcidin levels were significantly elevated in HD patients compared to control subjects and CKD patients. Prohepcidin showed statistically significant direct correlation with IL-6, CRP and serum creatinine in the whole patient group. In CKD group urinary hepcidin correlated positively to CRP. In the HD group prohepcidin correlated directly to serum creatinine. These results suggest that dialysis therapy is associated with elevated pro-hepcidin levels and directly related to inflammation (CRP&IL-6) and renal function parameters (serum creatinine). It is clear that hepcidin accumulates in renal insufficiency. Malyszko et al., (2005) reported a significant correlation between hepcidin and kidney function [23]. In addition, it was reported that hepcidin is also detectable in urine, further supporting the hypothesis that the kidney may be involved in the elimination of hepcidin [4].

In this study we observed increased levels of IL-6 and CRP with progress of renal function deterioration. IL-6 showed statistically significant direct correlation to urinary hepcidin, CRP and creatinine. CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes. CRP is a non specific but sensitive marker of inflammation. High CRP Levels show good correlation with the presence of an inflammatory process [23].

Synthesis of hepcidin is increased by inflammation, and decreased by iron deficiency, anemia and erythropoietin. Thus, dialysis patients frequently suffer the effects of both hepcidin increasing and decreasing factors [24]. This peptide can block iron absorption and release from both the liver and macrophages. The decreased availability of iron for erythropoiesis leads to the anemia of chronic disease or, in HD patients, aggravates an already existing anemia. HD is now widely considered an inflammatory state probably accounting for the increased serum hepcidin levels that have been associated with it [25]. Correlations of hepcidin with inflammatory markers in CKD have also been found in other studies, although not all. It therefore seems that diminished renal clearance and increased inflammation in the setting of renal failure may result in elevated serum hepcidin in patients with CKD. Future studies to assess the impact of residual renal function and clearance by dialysis on hepcidin levels are needed [8].

Dynamic evaluation of iron status is difficult in HD patients and can be further complicated by the presence of an inflammatory state. Several studies have proved that all parameters of iron balance (serum iron, TSAT, percent hypochromic RBCs and Hb concentration in reticulocytes) were clearly affected by the presence of an inflammatory state. It is confirmed that measurement of CRP must be part of the routine hematological assessment of hemo-dialyzed patients to allow the correct interpretation of data in anemia treatment [25].

In the present study we found that IL-6 and IFN-β levels were increased in CKD patients compared to controls, although not statistically significant, while both cytokines increased significantly in HD group when compared to control subjects. Hb was also inversely correlated to IFN-β and serum iron decreased with disease progress towards HD dependence. Hypoferremia is a common response to systemic infections or generalized inflammatory disorders. In mouse models, the development of hypoferremia during inflammation requires hepcidin, but the inflammatory signals that regulate hepcidin are largely unknown. Studies in human liver cell cultures, mice, and human volunteers indicate that IL-6 is the necessary and sufficient cytokine for the induction of excess hepcidin during inflammation and that the IL-6–hepcidin axis is responsible for the hypoferremia of inflammation [26]. Hepcidin in turn stops ferroportin from releasing iron stores. Inflammatory cytokines also appear to affect other important elements of iron metabolism, including decreasing ferroportin expression, and probably directly blunting erythropoiesis by decreasing the ability of the

bone marrow to respond to erythropoietin [27]. Interferon-β and lipopolysaccharide also down-regulate the expression of the macrophage iron transporter ferroportin 1, thus inhibiting iron export from macrophages, a process that is also affected by hepcidin. In addition, the limited availability of iron and the decreased biologic activity of erythropoietin lead to inhibition of erythropoiesis and the development of anemia. In summary, these mechanisms lead to a decreased iron concentration in the circulation and thus to a limited availability of iron for erythroid cells [28]. Studies of humans with chronic infections and severe inflammatory disease have shown markedly increased levels of hepcidin, strongly suggesting that elevated hepcidin levels play a key role in the anemia of inflammation and reticuloendothelial blockade [29].

Measurements of urinary hepcidin are possible in CKD patients with residual renal function. About 95% of hepcidin is retained in the kidney either because it is not freely filtered through the glomerular membrane and/or because it is reabsorbed and degraded in the proximal tubules, like other small peptides. Although we found significant correlations between urinary hepcidin and serum prohepcidin concentrations in our patients, urinary hepcidin concentrations may not accurately reflect the serum concentrations of hepcidin in kidney diseases. Since urinary secretion of hepcidin will depend on glomerular filtration and tubular reabsorption. Furthermore hepcidin mRNA has been detected in the kidney, which suggests the potential for local production and release into urine [16,30].

In this study, prohepcidin levels correlated positively with IL-6, markers of inflammation (CRP) and serum creatinine, but not with ferritin. Several studies proved strong correlation between hepcidin and ferritin [8,12]. Hepcidin and serum ferritin respond similarly to inflammation and changes in iron stores, and this is reflected in the strong correlation between hepcidin and ferritin in healthy volunteers. However, hepcidin responses take place on the time scale of a few hours, whereas changes in ferritin concentrations are much slower. Moreover, hepcidin levels were also higher in patients with normal ferritin levels, suggesting accumulation of hepcidin in renal insufficiency. Hepcidin was removed during dialyses in some but not all patients. The cause of this variability remains unclear, but might be attributed to differences in the membrane of the artificial kidney, residual renal function or induction of hepcidin by the HD procedure itself [16,31]. Scientific data indicated that hepcidin levels were approximately two- to threefold higher in the patients than in controls (depending on the hepcidin assay used) [8]. These figures must be compared with the 20- to 30-fold increase of serum β2-microglobulin in dialysis patients, a low-molecular-weight protein the excretion of which is almost completely governed by glomerular filtration. Thus, glomerular filtration has a limited influence on hepcidin levels that theoretically might be explained by the existence of a thus far unknown circulating hepcidin carrier that could serve as a mechanism to slow down the renal clearance of hepcidin. Alternatively, increased circulating levels of hepcidin may decrease hepatic hepcidin production through a feedback mechanism [16,31].

Multivariate regression analysis showed that prohepcidin was mostly predicted by both CRP and creatinine. Because of its renal elimination and regulation by inflammation, it is possible that progressive renal insufficiency leads to altered hepcidin metabolism, subsequently affecting enteric absorption of iron and the availability of iron stores. Thus, hepcidin likely plays a major role in the anemia of CKD as well as ESAs resistance [8].

Hb showed statistically significant inverse correlation to sTfR and sTfR/log ferritin ratio, while sTfR was inversely correlated to ferritin. Comparing the variables predicting the presence of ID between the two subgroups (ACD &pure ID) revealed that sTfR, sTfR/ log ferritin ratio, ferritin showed a statistically significant difference. The present data suggests that sTfR levels along with the sTfR/log ferritin ratio can be very useful in differentiating pure IDA, ACD and ACD with coexisting iron deficiency, thus providing a noninvasive alternative to bone marrow iron. This was on line with studies evaluating the importance of these parameters in this respect [21].

It could be concluded that increased hepcidin across the spectrum of CKD may contribute to abnormal iron regulation and erythropoiesis and may be a novel biomarker of iron status and erythropoietin resistance. The dialysis therapy is associated with elevated pro-hepcidin levels and is directly related to CRP, IL-6, and creatinine. Evaluation of human serum hepcidin should be useful in improving our understanding of the pathogenic role of hepcidin in various iron disorders, and in the development of appropriate therapeutic interventions. Large-scale human studies will be required to establish the utility of serum hepcidin measurements in the diagnosis and clinical management of iron disorders. If hepcidin proves to be a useful marker of responses to ESA and iron treatment, then cheaper methods, which can be reliably transferred to, and reproduced in, laboratories throughout the world, will be needed to facilitate the routine measurements that clinicians need.

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