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Is hepcidin related with anemia and bone mineral metabolism in children with non-dialysis chronic kidney disease?

Diyaliz gereksinimi olmayan kronik böbrek hastalıklı çocuklarda hepsidin ile anemi ve kemik mineral metabolizması ilişkili midir?

Osman Yeşilbaş[1], Nurdan Yıldız[2], Özgür Baykan[3], Harika Alpay[2]

[1]Department of Pediatrics, Marmara University Faculty of Medicine, İstanbul, Turkey
[2]Division of Pediatric Nephrology, Departmant of Pediatrics, Marmara University Faculty of Medicine, İstanbul, Turkey
[3]Department of Biochemistry, Marmara University Faculty of Medicine, İstanbul, Turkey

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Abstract

Aim: Functional iron deficiency secondary to inflammation and increased serum hepcidin lead to erythropoietin-resistant anemia in children with chronic kidney disease. Vitamin D deficiency, parathyroid hormone, and phosphate can also participate in chronic inflammation and anemia. The aim of this study was to evaluate the association between hepcidin, bone mineral metabolism, and anemia in non-dialysis pediatric patients with chronic kidney disease.

Material and Methods: Thirty-five patients with stage 2–4 chronic kidney disease and 35 healthy subjects were enrolled in the study. Serum creatinine, blood urea nitrogen, uric acid, C-reactive protein, interleukin-6, hepcidin, complete blood count, ferritin, calcium, phosphorus, parathyroid hormone, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and fibroblast growth factor-23 levels were compared between the groups.

Results: Ferritin, C-reactive protein, interleukin-6, blood urea nitrogen, creatinine, uric acid levels, and percentages of reticulocytes were significantly higher than in the controls ($p<0.05$). The mean serum hepcidin levels in the chronic kidney disease and control groups were 9.6±5.2 (range, 2.15–25.3) and 9.7±4.3 (range, 3.4–22.2) ng/mL and were not significantly different in either group. There were no differences in terms of serum phosphorus, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and fibroblast growth factor-23 levels between the groups ($p>0.05$). Serum hepcidin levels were not correlated with anemia parameters, serum fibroblast growth factor-23, phosphorus, uric acid, C-reactive protein, parathyroid hormone, and 25-hydroxyvitamin D levels ($p>0.05$). However, serum hepcidin levels were correlated with 1,25-dihydroxyvitamin D and interleukin-6 levels ($p=0.013$ and $p=0.002$, respectively).

Öz

Amaç: İnflamasyona ikincil fonksiyonel demir eksikliği ve artmış hepsidin düzeyleri kronik böbrek hastalığı olan çocuklarda eritropoetin dirençli anemiye neden olmaktadır. Vitamin D eksikliği, parathormon ve fosfor da kronik inflamasyon ve anemiye katkıda bulunabilmektedir. Bu çalışmanın amacı, diyaliz ihtiyacı olmayan kronik böbrek hastalıklı çocuklarda hepsidin ile anemi ve kemik mineral metabolizmasının ilişkisini araştırmaktır.

Gereç ve Yöntemler: Evre 2–4 kronik böbrek hastalığı olan 35 çocuk ile 35 sağlıklı çocuk çalışmaya alındı. Serum kreatinin, kan üre nitrojeni, ürik asit, C-reaktif protein, interlökin-6, hepsidin, tam kan sayımı, ferritin, kalsiyum, fosfor, parathormon, 25-hidroksivitamin D, 1,25- hidroksivitamin D ve fibroblast büyüme faktörü-23 düzeyleri iki grup arasında karşılaştırıldı.

Bulgular: Ferritin, C-reaktif protein, kan üre nitrojeni, kreatinin ve ürik asit düzeyleri ile retikülosit yüzdesi kontrol grubuna göre anlamlı yüksek idi ($p<0,05$). Kronik böbrek hastalığı olan grup ile sağlıklı çocuklardan oluşturulan grupta, ortalama serum hepsidin düzeyleri sırası ile 9,6±5,2 (en düşük-en yüksek, 2,15–25,3) ve 9,7±4,3 (en düşük-en yüksek, 3,4–22,2) ng/mL olup iki grup arasında anlamlı fark saptanmadı. İki grup arasında serum fosfor, 25-hidroksivitamin D, 1,25- hidroksivitamin D ve fibroblast büyüme faktörü-23 düzeyleri bakımından da anlamlı farklılık saptanmadı ($p>0,05$). Hasta grubundaki serum hepsidin düzeyi ile anemi belirteçleri, fibroblast büyüme faktörü-23, fosfor, ürik asit, C-reaktif protein, 25-hidroksivitamin D ve parathormon düzeyleri korele değildi ($p>0,05$). Bununla birlikte serum hepsidin düzeyleri ile 1,25- hidroksivitamin D ve interlökin-6 düzeylerinin korelasyon gösterdiği görüldü (sırasıyla $p=0,013$ ve $p=0,002$).

Corresponding Author /Sorumlu Yazar: Osman Yeşilbaş E-mail /E-posta: drosmanyesilbas@gmail.com
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Conclusion: Serum hepcidin levels may not increase significantly in non-dialysis pediatric patients with chronic kidney disease despite high levels of inflammatory markers such as C-reactive protein and interleukin-6. The increase of serum hepcidin levels may be inhibited by effective treatment of anemia with iron supplementation and erythropoietin, and the treatment of secondary hyperparathyroidism with phosphate binders and the active form of vitamin D, which decrease serum parathyroid hormone and fibroblast growth factor-23 levels, and control inflammation to some extent.

Keywords: Anemia, children, chronic kidney disease, hepcidin, vitamin D

Çıkarımlar: Diyaliz ihtiyacı olmayan kronik böbrek hastalıklı çocuklarda, C-reaktif protein ve interlökin-6 gibi inflamasyon belirteçlerindeki artışa rağmen serum hepsidin düzeyleri anlamlı olarak artmayabilir. Demir desteği ve eritropoetin ile etkin anemi tedavisi ile fosfat bağlayıcılar ve aktif vitamin D ile ikincil hiperparatiroidizmin etkin tedavi edilerek serum parathormon ve fibroblast büyüme faktörü-23 düzeylerinin düşürülmesi bu hastalardaki inflamasyonu kontrol altına alarak serum hepsidinin artmasını engelleyebilir.

Anahtar sözcükler: Anemi, çocuklar, hepsidin, kronik böbrek hastalığı, vitamin D

Introduction

Anemia is common in patients with chronic kidney disease (CKD) and contributes to adverse outcomes such as cardiovascular disease and death. The etiology of anemia in CKD is multifactorial. Decreased production of erythropoietin (EPO) secondary to impaired renal function is the main cause. However, CKD-associated anemia can be resistant to EPO, mostly due to functional iron deficiency secondary to non-infective causes of inflammation (1).

Hepcidin, an acute-phase protein produced in the liver, is a negative regulator of iron use, and crucial in the handling of iron availability for erythropoiesis. Increased hepcidin levels in patients with CKD are supposed to be responsible for functional iron deficiency and contribute to renal anemia and resistance to erythropoiesis-stimulating agents (ESA) (2). Hepcidin secretion is inhibited in anemia and hypoxia, whereas it is stimulated by iron loading and inflammation in general, and in particular with the inflammatory cytokine interleukin-6 (IL-6). Additionally, lower hepcidin clearance and CKD-related inflammation tend to increase hepcidin levels in these patients (1–5).

In patients with CKD, vitamin D deficiency, and high intact parathyroid hormone (iPTH) and phosphate levels may contribute to chronic inflammation and increase of hepcidin, which consequently lead to ESA-resistance anemia due to the decreased bone marrow production of red blood cells (RBCs) (6–10). Fibroblast growth factor 23 (FGF23) is a phosphate-regulating hormone primarily secreted by osteocytes and osteoblasts. It is increased in CKD secondary to high phosphate levels and leads to increase in iPTH indirectly by decreasing 1,25-dihydroxy vitamin D [1,25$(OH)_2$D] synthesis. It decreases the phosphate reabsorption in renal proximal tubules and inhibits absorption of phosphate in the intestine by reducing active vitamin D synthesis (11, 12).

There is a limited number of studies that evaluate the relation between bone mineral metabolism, inflammation, anemia, and hepcidin in non-dialysis patients with CKD. Most of these studies were performed in adult patients, and there are insufficient data to evaluate this relation in pediatric patients with CKD.

The aim of this study was to evaluate the relationship between anemia, inflammatory markers, bone mineral metabolism, and hepcidin in children with non-dialysis CKD.

Material and Methods

Thirty-five children (17 girls, 18 boys) with stage 2–4 CKD who were followed up at Marmara University School of Medicine Hospital Pediatric Nephrology Outpatient Clinic, and 35 (20 girls, 15 boys) healthy children were included in the study. After a full explanation of the study was given to the parents and children, informed consent was obtained from all participants prior to the testing process. The study was approved by the Ethics Committee of Marmara University School of Medicine (MAR-AEK-09-2012-0188). This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The demographic data and medical therapy of the patients were recorded from the medical files. Chronic kidney disease was classified into five stages according to the National Kidney Foundation guidelines (13). Estimated glomerular filtration rate (GFR) was calculated from height and plasma creatinine (Cr) using the original Schwartz formula (14).

None of the children in the study group had acute or chronic infection and inflammation, uncontrolled hypertension, thrombosis, and received any antibiotics, anti-inflammatory, and antiproteinuric drugs, intravenous iron therapy or transfusion within four weeks before enrollment. The dosages of recombinant EPO (rhEPO) and iron supplementation had been stable for at least four weeks. All rhEPO supplements were in the form of recombinant epoetin alfa (Eprex®, Santa Farma). Children in the control group had normal renal function as defined as a GFR of over 90 mL/min/1.73m^2. Children in the control group

who used vitamin D, oral calcium or iron supplementation in the last four months were excluded.

In all patients and controls, fasting morning samples were obtained, plasma and sera were separated by centrifugation, and stored at -20°C until required for testing. Complete blood count, blood urea nitrogen (BUN), serum Cr, uric acid, calcium, phosphorus, alkaline phosphatase (ALP), high sensitivity C-reactive protein (hs-CRP), IL-6, percentages of reticulocyte, iron, total iron-binding capacity (TIBC), transferrin saturation (TSAT), ferritin, iPTH, 25-hydroxy vitamin D [25(OH)D], 1,25$(OH)_2$D, FGF-23, and hepcidin-25 levels were measured and the results of two groups were compared.

Serum BUN, uric acid, iron, TIBC, calcium, phosphorus, ALP, hs-CRP, and urine Cr concentrations were analyzed by automated spectrophotometric method (Cobas 8000 Modular Analytics, Roche Diagnostics, Germany). Serum Cr concentration was measured using the calorimetric Jaffe method. Complete blood count and percentages of reticulocytes were performed using an automated hematology analyzer (LH 780, Beckman Coulter, USA). Ferritin and iPTH were measured using an electrochemiluminescence immunoassay (Modular Analytics E170, Roche Diagnostics, Germany). Serum vitamin D concentrations were studied using high-performance liquid chromatography, which measured both D2 and D3, and is reported as serum 25 (OH)D concentration. 1,25$(OH)_2$D levels were measured using an enzyme-linked immunosorbent assay (ELISA).

Serum IL-6 levels were studied using a solid-phase enzyme-labeled chemiluminescent method (Immulite 2000, Siemens, Germany). Serum hepcidin-25 levels and FGF-23 were measured using ELISA with a commercially available kit (Human Hepc-25 ELISA Kit, EIAab Science, Wuhan, China). Hepcidin assay range was 1.56–100 ng/mL. Intra-assay coefficient of variations (CV) for low, mid-range, and high-level human hepcidin-25 were 6.29%, 5.71%, and 3.07%, respectively. Inter-assay CV for low, mid-range, and high-level human hepcidin-25 were 4.98%, 5.27%, and 3.53%, respectively. Serum intact FGF-23 levels were measured using ELISA (FGF-23 ELISA Kit, Eastbiopharm, China). The FGF-23 assay range was 5–1500 pg/mL. The intra-assay and inter-assay CV for FGF-23 were <10% and <12%, respectively.

Statistical Analysis

All data were analyzed using the Statistical Packages for the Social Sciences (SPSS Inc., Chicago, IL, USA) Ver. 21.0 software package. A one-sample Kolmogorov-Smirnov test was used to determine the normality of the data. Data are expressed as mean±standard deviation (SD). Data that were not distributed normally are expressed as median (interquartile range, IQR). Differences between the groups were analyzed using the Mann-Whitney U test. The association between variables was analyzed using Spearman's correlation tests in each group. A p value of <0.05 was regarded as statistically significant.

Table 1. Demographic data of the patients and controls

	Patients n=35	Controls n=35
Age (years±SD)	12.3±4.5	11.7±4.3
Sex (F/M)	17/18	20/15
Primary disease		
CAKUT	25 (71.4%)	
Glomerulonephritis	4 (11.4%)	
Hereditary disease	6 (17.2%)	
CKD stage		
Stage 2	8	
Stage 3	13	
Stage 4	14	
Medication		
Oral iron	29	
rhEPO	3	
Oral phosphorus binders	12	
25(OH)D	35	
1,25$(OH)_2$D	15	

SD: Standard deviation; CAKUT: Congenital anomalies of the kidneys and urinary tract; CKD: Chronic kidney disease; F: Female; M: Male; rhEPO: Recombinant erythropoietin; 1,25$(OH)_2$D: 1,25-dihydroxyvitamin D; 25(OH)D: 25-hydroxyvitamin D

Results

The mean ages of the CKD group (17 females, 18 males) and controls (20 females, 15 males) were 12.3±4.5 (range, 5–18) and 11.7±4.3 (range, 5–18) years, respectively. The most common underlying cause of CKD was congenital anomalies of the kidneys and urinary tract (CAKUT) (71.4%) in 25 patients. Demographic data and the medications of the patients are presented in Table 1.

The laboratory investigations of the patients and controls are shown in Table 2. In the CKD group, hemoglobin (Hb), hematocrit (Hct), TIBC, and ferritin levels were significantly different than those in the control group ($p<0.05$) (Table 2). The mean Hb level of the patients with CKD was 12.5±1.7 (range, 7.7–16.9) g/dL. In the CKD group, only one patient had an Hb level lower than 10 g/dL. Twenty-six of the CKD group had Hb levels higher than 12 g/dL. Only three patients were on EPO treatment and 29 patients were taking oral iron therapy.

Table 2. Laboratory parameters of the patients and controls

Parameters	Patients (n=35)	Controls (n=35)	p
GFR (mL/min/1.73 m^2) (mean±SD)[a]	41.8±21	113.5±11.9	<0.001
Cr (mg/dL) [median (IQR)][b]	1.5 (0.72)	0.5 (0.2)	<0.001
Hgb (g/dL) (mean±SD)[a]	12.5±1.7	13.4±1.2	0.011
Hct (%) (mean±SD)[a]	36.3±5.1	38.6±3.2	0.012
Iron (ug/dL) [median (IQR)][b]	77 (58)	98 (38)	0.359
TIBC (µg/dL) (mean±SD)[a]	304.3±60.4	398.9±40.9	<0.001
Ferritin (ng/mL) [median (IQR)][b]	95.5 (144.3)	23.4 (16.1)	<0.001
TSAT (%) [median (IQR)][b]	31 (25)	24 (14)	0.136
Reticulocytes (%) [median (IQR)][b]	1.1 (0.6)	0.93 (0.71)	0.031
IL-6 (pg/mL) (mean±SD)[a]	3.08±2.33	2.42±1.76	0.047
hs-CRP (mg/L) [median (IQR)][b]	1 (1.7)	0.6 (0.6)	0.016
Uric acid (mg/dL) (mean±SD)[a]	5.6±1.5	3.6±1	<0.001
Calcium (mg/dL) [median (IQR)][b]	9.5 (0.6)	9.7 (0.4)	0.002
Phosphorus (mg/dL) (mean±SD)[a]	4.7±0.7	4.7±0.8	0.977
iPTH (pg/mL) [median (IQR)][b]	111.2 (147)	39.9 (19.5)	<0.001
Hepcidin-25 (ng/mL) (mean±SD)[a]	9.6±5.2	9.7±4.3	0.698
$25(OH)_2D$ (ug/L) [median (IQR)][b]	19 (18.9)	17.5 (10.8)	0.094
$1,25(OH)_2D$ (pmol/L) [median (IQR)][b]	123.3 (263.2)	103 (251.5)	0.635
FGF-23 (pg/mL) [median (IQR)][b]	480.6 (524.4)	370.5 (583.2)	0.916

*Mann-Whitney U test; [a]Student's t-test; [b]Mann-Whitney U test; CKD: Chronic kidney disease; GFR: Glomerular filtration rate; BUN: Blood urea nitrogen; Cr: Creatinine; Hgb: Hemoglobin; Hct: Hematocrit; TIBC: Total iron-binding capacity; TSAT: Transferrin saturation; IL-6: Interleukin-6; hs-CRP: High sensitive C-reactive protein; iPTH: Parathyroid hormone; $25(OH)_2D$: 25-hydroxyvitamin D; $1,25(OH)_2D$: 1,25-dihydroxyvitamin D; FGF-23: Fibroblast growth factor-23; SD: Standard deviation; IQR: Interquartile range

Ferritin, hs-CRP, IL-6, BUN, Cr, uric acid levels, and percentages of reticulocytes were significantly higher in the CKD group than in the controls (p<0.05) (Table 2). The median (IQR) of iPTH levels in the patients and controls were 111.2 (147) (range, 18.9–354.6) and 39.9 (19.5) (range, 15.9–81.8) pg/mL, respectively (p<0.001). The iPTH levels were less than 200 pg/mL in 26 patients, 10 of whom had normal iPTH levels. The mean serum phosphorus levels were 4.7±0.7 (range, 3.6–6.6) mg/dL in the CKD group and 4.7±0.8 (range, 3.4–6.3) mg/dL in the controls. Serum phosphorus levels were not different in between the patients and controls (p= 0.977), whereas serum calcium was significantly lower in the patients with CKD (Table 2).

The mean serum hepcidin-25 levels in the CKD and control groups were 9.6±5.2 (range, 2.15–25.3) and 9.7±4.3 (range, 3.4–22.2) ng/mL, and were not significantly different in either group. In addition, serum iron, $25(OH)_2D$, $1,25(OH)_2D$, FGF-23 levels, and TSAT were not different between the study groups (p>0.05) (Table 2).

The hepcidin-25, FGF-23, uric acid, and IL-6 levels of the patients were not correlated with Hb and Htc levels. There were significant correlations between Htc, TIBC, TSAT, and hs-CRP. Serum hepcidin-25 levels were significantly correlated with $1,25(OH)_2D$, IL-6, and calcium levels (p=0.013, p=0.02, and p=0.041 respectively). However, no significant relation was found between hsCRP, uric acid, FGF-23, phosphorus, iPTH, 25(OH)D, and hepcidin-25 (p>0.05) (Table 3).

Discussion

Anemia develops gradually during progressive decline in renal function in children and adults with CKD. Erythropoietin deficiency and disordered iron homeostasis are key features of the anemia in CKD (15). Hepcidin was established as a key regulator of iron homeostasis and involved in the pathogenesis of anemia of chronic disease (16). Hepcidin levels have been found to be elevated in both adults and children with CKD who are on dialysis. Hepcidin homeostasis is regulated by renal clearance, iron status, erythropoiesis, and an ESA treatment regimen in patients with CKD (17, 18). Several studies have shown this relationship between ESA and hepcidin in patients undergoing dialysis (19, 20). An inverse correlation between

Table 3. Spearman's correlation tests between anemia parameters, bone mineral metabolism and inflammation markers of the patients and controls

Parameters			Hepcidin (ng/mL)	hs-CRP (mg/L)	IL-6 (pg/mL)	FGF-23 (pg/mL)
Hepcidin (ng/mL)	Patients	r		0.004	0.508	0.323
		p		0.984	**0.02***	0.059
	Controls	r		0.07	-0.222	0.080
		p		0.690	0.900	0.648
Hgb (g/dL)	Patients	r	0.028	0.105	0.080	0.093
		p	0.875	0.548	0.648	0.59
	Controls	r	-0.63	0.141	-0.233	0.159
		p	0.719	0.419	0.178	0.361
Iron (ug/dL)	Patients	r	-0.009	-0.325	-0.111	0.079
		p	0.960	0.057	0.524	0.653
	Controls	r	0.113	-0.316	-0.234	0.15
		p	0.519	0.065	0.177	0.391
Ferritin (ng/mL)	Patients	r	-0.163	-0.121	0.477	0.014
		p	0.292	0.490	**0.004***	0.936
	Controls	r	0.045	0.006	-0.058	0.213
		p	0.797	0.972	0.743	0.218
TSAT (%)	Patients	r	-0.038	-0.417	-0.165	0.068
		p	0.829	**0.013***	0.343	0.699
	Controls	r	-0.001	-0.298	-0.344	0.121
		p	0.996	0.082	**0.043***	0.487
Calcium (mg/dl)	Patients	r	-0.347	0.224	0.069	-0.04
		p	**0.041***	0.196	0.692	0.983
	Controls	r	-0298	0.105	-0.035	0.05
		p	0.082	0.55	0.843	0.773
Phosphorus (mg/dL)	Patients	r	-0.213	-0.041	-0.211	-0.158
		p	0.220	0.815	0.224	0.363
	Controls	r	0.082	-0.106	0.246	-0.410
		p	0.055	0.543	0.154	**0.012***
iPTH (pg/mL)	Patients	r	0.156	0.022	-0.012	-0.084
		p	0.372	0.902	0.946	0.630
	Controls	r	0.19	-0.134	0.046	0.05
		p	0.273	0.442	0.791	0.777
25(OH)D (ug/L)	Patients	r	-0.125	-0.162	-0.142	-0.81
		p	0.474	0.354	0.415	0.643
	Controls	r	-0.110	-0.125	-0.233	-0.112
		p	0.528	0.474	0.178	0.523
1,25(OH)2D (pmol/L)	Patients	r	0.421	0.201	-0.214	0.933
		p	**0.013***	0.255	0.225	**<0.001***
	Controls	r	0.076	-0.170	-0.44	0.904
		p	0.665	0.330	0.801	**<0.001***
FGF-23 (pg/mL)	Patients	r	0.323	0.143	-0.106	
		p	0.059	0.413	0.544	
	Controls	r	0.080	-0.042	-0.157	
		p	0.648	0.812	0.367	

*p<0.05. FGF-23: Fibroblast growth factor-23; IL-6: Interleukin-6; Hgb: Hemoglobin; hs-CRP: High sensitive C-reactive protein; iPTH: Parathyroid hormone; TSAT: Transferrin saturation; $1,25(OH)_2D$: 1,25-dihydroxyvitamin D; $25(OH)_2D$: 25-hydroxyvitamin D

serum hepcidin levels and EPO doses and a decline in the hepcidin level after the start of EPO therapy was reported (21).

In our study, hepcidin levels in the CKD group were not different than the controls. In addition, hepcidin was not correlated with anemia parameters such as ferritin, TSAT, iron, and Hb levels. Despite the fact that most studies reported increased hepcidin levels in patients with CKD, Kulaksiz et al. (22) observed no correlation between prohepcidin, iron status, ferritin, and TSAT, similar to our study. Our study group consisted of patients with early-stage CKD and Hb levels were higher than 12 g/dL in 26 patients. Twenty-nine of our patients with CKD were receiving oral iron and only three were receiving EPO therapy. Although this is a cross-sectional study, we may speculate according to our results that the elevation of serum hepcidin levels may be inhibited by effective treatment of anemia in the early course of CKD.

Hepcidin synthesis is markedly increased during infection and inflammation, and reduces serum iron (2). Patients with CKD have a chronic inflammatory state. A declining renal function may affect the levels of additional inflammatory molecules, such as serum hs-CRP or IL-6. The relationship between hepcidin and inflammatory mediators has not been consistently demonstrated in patients with CKD (1).

Malyszko and Ganz et al. (1, 23) demonstrated a correlation between hepcidin and CRP. By contrast, many studies have not reported this correlation (24). According to numerous studies, IL-6 is apparently a key inducer of hepcidin synthesis during inflammation, whereas IL-1 or tumor necrosis factor alpha (TNF-α) did not affect hepcidin synthesis (1, 5). On the other hand, Camaschella et al. (25) reported that inflammatory cytokines, namely IL-6, IL-1, TNF-α, and gamma-interferon were involved in activation of hepcidin production and found that IL-6 was the most important cytokine in the pathogenesis of the anemia of chronic disease.

Similarly, we found increased levels of inflammatory molecules of IL-6, hs-CRP, and uric acid in patients with CKD. Interleukin-6 was correlated with hepcidin levels, whereas no correlation was observed between hs-CRP, uric acid, and hepcidin. However, hepcidin levels in the CKD group were not different than the controls. None of our patients had additional acute or chronic inflammatory conditions other than CKD in the study group. According to our results, we suggest that inflammation of CKD may not show a marked effect on serum hepcidin levels if there are no concomitant acute or chronic inflammatory conditions. The etiology of CKD may also play a role in inflammatory situation. In the majority of our patients, the underlying cause of CKD was CAKUT. Therefore, chronic inflammation may be less important in patients with CAKUT in the early stages of CKD. We could not perform statistical analysis according to the etiology of CKD because we had a small number of patients.

It has also been reported that low Hb levels were accompanied by higher levels of both inflammatory markers of IL-6 and CRP in patients undergoing hemodialysis, and IL-6 was found to antagonize the response to EPO (26). However, we found no relation between anemia and the inflammatory markers of IL-6, hs-CRP, and uric acid, other than a weak correlation between Hct and hs-CRP. The lack of correlation between inflammatory markers and anemia in our results can be explained by the fact that good anemia control was achieved in the early stages of CKD.

Recent studies suggested that vitamin D deficiency might play an important role increasing hepcidin levels and correlated inversely with the ESA-resistance anemia in patients with CKD (7, 9). Vitamin D has been shown to decrease circulating hepcidin levels in healthy volunteers and patients undergoing hemodialysis (27). Increased iPTH has a similar effect on hepcidin. Treatment with vitamin D and calcitriol was reported to suppress inflammation and improve the effectiveness of EPO therapy in healthy adults and patients with CKD (7). Bacchetta et al. (8) showed that $1,25(OH)_2D$ directly inhibited hepcidin synthesis by binding to a receptor in the hepcidin gene encoding HAMP (hepcidin antimicrobial peptide). In our study, serum hepcidin, 25(OH)D, $1,25(OH)_2D$, iron, and TSAT levels were not different between the study groups, and serum hepcidin levels were significantly correlated with $1,25(OH)_2D$. Although hs-CRP, IL-6, and iPTH were significantly higher in the CKD group than in the controls, we suggest that effective and early vitamin D treatment may suppress hepcidin synthesis by the mechanisms mentioned above.

It has been reported that hyperphosphatemia might play an important role in the development of chronic inflammation in CKD (7). Increased FGF-23 levels are common in CKD, likely as a physiologic adaptation to maintain normal serum phosphorus concentrations. In patients with CKD, regulation of FGF-23 remains unclear, particularly in the early stages of the disease, where FGF-23 concentrations are elevated before the increase in serum phosphorus (12). However, the association between FGF-23 and inflammation in CKD remains unclear. Munoz Mendoza et al. (11) showed that high levels of FGF-23 were independently associated with inflammation in patients with CKD. In contrast, Braithwaite et al. (28) found no correlations between FGF-23 and inflammatory parameters. A few human studies suggested a possible association between FGF-23 and anemia in patients with CKD (29). It has also been reported that improvements in iron status with iron supplementation were associated with a significant decrease in FGF-23 concentrations (28). In our study, serum phosphorus and FGF-23 were not different between the study groups. Dietary phosphate restriction and good drug compliance may explain why serum phosphate levels in the patient group were not different from the controls. According to these findings, we may suggest that good control of serum phosphate concentration with oral phosphate binding agents prevents the increase of FGF-23 levels, and may contribute to suppressing inflammation and hepcidin synthesis in children with CKD. In our study, although the level of FGF-23 and $1,25(OH)_2D$ were higher in the CKD group than in the healthy group, the difference was only slightly significant ($p>0.05$). In our opinion, initiation of the vitamin D treatment in the early period was the reason for its high level that was found in the CKD group. We think that the unexpected result of the positive correlation between FGF-23 and $1,25(OH)_2D$ found in our study was due to our vitamin D treatment along with FGF-23 increasing in the early period of CKD. Additionally, we believe that in comparison to the healthy group, the significant increase of FGF-23 in the CKD group was precluded by the suppression of inflammation with vitamin D supplementations.

Although our study has potential limitations due to its cross-sectional design and small sample size, to our knowledge, this is the first study to evaluate the relation between bone-mineral metabolism, anemia, inflammation, and hepcidin in children with non-dialysis CKD. A relatively small number of patients is an inherent problem for most single-center studies in the pediatric CKD population. As a result of our study, it is important to note that hepcidin levels in pediatric patients with CKD may not increase if hyperphosphatemia and hyperparathyroidism are well controlled, and therefore we think that our results are considerable for the discussion of the rela-

tion between hepcidin and bone mineral metabolism. We hope our work paves the way for large prospective studies in pediatric patients with CKD.

In conclusion, our data suggest that serum hepcidin levels are not elevated significantly in non-dialysis pediatric patients with CKD despite a mild increase of inflammatory markers such as hs-CRP and IL-6. The increase of serum hepcidin levels may be inhibited by effective treatment of anemia and secondary hyperparathyroidism with phosphate binders and the active form of vitamin D, which decrease serum iPTH, FGF-23, and phosphorus levels, and control inflammation to some extent.

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