Relationship Between Prohepcidin and Cardiovascular Risk Markers in End Stage Renal Failure Patients

Son Dönem Böbrek Yetersizlikli Hastalarda Prohepsidin ve Kardiyovasküler Risk Belirteçleri Arasındaki İlişki

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ABSTRACT

OBJECTIVE: The leading cause of death in patients with end-stage renal disease (ESRD) is cardiovascular disease. Prohepcidin, which is the precursor of hepcidin is a peptide hormone produced by the liver, and appears to be the master regulator of iron homeostasis in humans. High prohepcidin levels may contribute to progression of cardiovascular disease in end-stage renal insufficiency patients. However, any such association remains poorly understood. The purpose of the present study was to investigate the relationship between prohepcidin and cardiovascular risk markers in end-stage renal failure patients.

MATERIAL and METHODS: Twenty-two chronic hemodialysis patients, 21 chronic peritoneal dialysis patients, and 16 healthy controls were included in the present study. The levels of serum prohepcidin (the precursor form of hepcidin), high sensitivity C-reactive protein (hs-CRP), troponin-T (TT), and cystatin C (CC) were determined using commercial kits. The left ventricular mass index (LVMI) was estimated using echocardiography.

RESULTS: The levels of the CVD risk markers TT, CC, and prohepcidin differed, with statistical significance, between the patient and control groups. Prohepcidin level was significantly associated with CC concentration (β=0.855, R2=0.73, p<0.001), TT level (β=0.456, R2=0.20, p=0.002), and the LVMI (β=0.435, R2=0.19, p=0.003). However, no significant association between prohepcidin level and hs-CRP concentration was evident (β=0.124, R2=0.015, p<0.42).

CONCLUSION: Our data suggest that the prohepcidin level can serve as a biomarker of cardiovascular disease. This level is closely associated with the cardiovascular risk markers of CC and TT concentrations, as well as the LVMI.

KEY WORDS: Cardiovascular risk, Hemodialysis, Peritoneal dialysis, Prohepcidin

ÖZ

AMAÇ: Hepsidinin öncül molekülü olan prohepsidin karaciğerden yapılan bir peptit hormon olup demir metabolizmasının ana düzenleyicisidir. Son dönem böbrek yetersizlikli hastalarda yüksek prohepsidin seviyelerinin kardiyovasküler hastalıklarla ilişkili olabileceği düşünülmektedir. Ancak bu ilişki net bir şekilde anlaşılamamıştır. Bu çalışmadaki amacımız, SDBY'li hastalarda prohepsidin ile kardiyovasküler risk belirteçleri arasında ilişkiyi araştırmaktır.

GEREÇ ve YÖNTEMLER: Çalışmaya 22 kronik hemodiyaliz, 21 kronik periton diyaliz hastası ile birlikte 16 sağlıklı kontrol dahil edildi. Rutin laboratuvar tetkiklerine ilave olarak hastalardan prohepsidin, yüksek hassasiyetli C reaktif protein (hsCRP), troponin T (TT) ve sistatin C (CC) değerleri ölçüldü. Ekokardiyografi ile sol ventrikül kitle indeksleri (LVMI) belirlendi.

BULGULAR: Hastaların olduğu gruplarda ölçülen prohepsidin, TT, CC yüksekliği kontrol grubuna göre istatistiksel olarak anlamlıydı. Ayrıca prohepsidin CC (β=0,855, R2=0,73, p<0,001), TT (β=0,456, R2=0,20, p=0,002), LVMI (β=0,435, R2=0,19, p=0,003) ile korelasyon göstermekteydi. HsCRP (β=0,124, R2=0,015, p<0,42) ile ilişki saptanmadı.

SONUÇ: Sonuçlarımız prohepsidinin de diğer kardiyovasküler belirteçlerle korelasyon gösterdiği ve bu hastalıkların tespitinde kullanılabileceğini düşündürmektedir.

ANAHTAR SÖZCÜKLER: Kardiyovasküler risk, Hemodiyaliz, Periton diyalizi, Prohepsidin

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in patients with chronic renal dysfunction (1,2). The mortality (overall death) rates from cardiac disease in end-stage renal disease (ESRD) patients are 20- to 30-fold higher than are those of age-, gender-, and race-matched controls and the risk factor is as high as 100-fold in younger patients (3, 4). Many factors, including low-grade inflammation, hypervolemia, and anemia, may increase the risk of CVD (5).

Hepcidin is synthesized as an 84 amino acid precursor, prohepcidin. After the 24 amino acid part is split off from the N terminal domain, 60 amino acid prohepcidin passes from the basolataral membrane of hepatocytes to the blood. Prohepcidin is processed by propeptide convertase which is thought to be located in bloodstream or capillary cell membrane. The mature form of the hormone is 20, 22 and 25 amino acid hepcidin peptides (6,7). Prohormone measurements are more commonly performed as mature hormone measurement is not standardized. Urine hepcidin level is positively correlated with hepatic hepcidin mRNA release (8). Hepsidin appears to be the master regulator of iron homeostasis in humans (9). Hepcidin is synthesized by hepatocytes in response to both iron overload and inflammatory stimuli (10). The hormone acts by downregulating both iron absorption and the release of iron from enterocytes and macrophages, in response to high iron levels and the synthesis of inflammatory cytokines (11). Recent studies suggest that hepcidin may play a role in the development of many significant adverse events including anemia, inflammation, and the generation of antimicrobial activity (12, 13). In patients with chronic renal failure, chronic systemic inflammation (which is common), low-level renal clearance, and insufficient dialysis may cause an increase in serum levels of prohepcidin, the precursor form of the hormone levels.

Hepcidin concentrations in humans correlate with serum iron levels and the concentrations of inflammation markers including IL-6 and high sensitivity C-reactive protein (hs-CRP) (14). Although an increased hepcidin level may serve as a marker of cardiovascular risk in ESRD patients, little is known about how hepcidin concentration is modulated in such patients (15).

In the present study, we sought principally to explore the relationship between cardiovascular risk indicators including the levels of hs-CRP, troponin-T (TT), cystatin C (CC) and hemoglobin; the left ventricular mass index (LVMI); and prohepcidin concentration, in dialysis patients. We also explored whether any of the observed correlations differed between hemodialysis and peritoneal dialysis patients.

MATERIAL and METHODS

Study Population

The present study was approved by Bursa Regional Ethics Committee and all subjects gave written informed consent. All procedures were in accordance with the Second Declaration of Helsinki.

A total of 43 ESRD patients undergoing continuous renal replacement therapy in our hospital and fulfilling the inclusion/exclusion criteria were included in the study. Twenty-two hemodialysis patients (group 1), 21 peritoneal dialysis patients (group 2), and 16 normal controls were selected to participate in the present study. A subject was eligible if that subject was aged 18 years or over. The exclusion criteria included pregnancy; the presence of malignancy or an acute inflammatory disease; current use of drugs (statins, non-steroidal anti-inflammatory agents, or immunosuppressors); the presence of liver or thyroid disease; hepatitis; dialysis adequacy (Kt/V<1.2 for hemodialysis, Kt/V<1.7 for peritoneal dialysis) and/or hemodynamic instability. A detailed medical history was obtained from each subject and a physical examination was performed.

The mean arterial pressure (MAP) was calculated using blood pressure measurements taken prior to dialysis, with each patient in the horizontal position. The following formula was used: MAP=diastolic pressure+ (systolic pressure-diastolic pressure/3).

Overall, the causes of ESRD were diabetes mellitus (n=1), chronic glomerulonephritis (n=9), presence of a polycystic kidney (n=7), hypertension (n=6), chronic pyelonephritis (n=5), tubular necrosis (n=2), Alport syndrome (n=3), Systemic lupus erythematosus (n=1), amyloidosis (n=1), and conditions of unknown etiology (n=8). The mean duration of dialysis treatment was 77.05±53.7 months (range: 9–240 months). Hemodialysis was performed three times a week for 4–5 h on average, using F6 polysulphone capillaries (Gambro Renal Products, Lakewood, CO). A bicarbonate dialysis solution was employed for the treatment of hemodialysis patients. Peritoneal dialysis patients received either automated peritoneal dialysis (APD) or standard continuous ambulatory peritoneal dialysis (CAPD).

Laboratory and Echocardiographic Methods

Venous blood samples were obtained from the antecubital region of all patients between 08.00-09.00 after an 8–12 hour overnight fast, prior to a dialysis session. Serum was obtained and all samples were frozen at –70°C if not analyzed immediately for prohepcidin. Serum glucose, urea, creatinine, uric acid, albumin, iron (Fe), iron-binding capacity (IBC), total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglyceride levels were determined in our central laboratory using a Roche P-Modular analyzer (Roche Diagnostics GmbH, Mannheim, Germany) employing Roche Diagnostics reagents. LDL-cholesterol levels were calculated using Friedewald's equation. The hematocrit, and hemoglobin (Hb), erythrocyte, lymphocyte, and thrombocyte counts, were determined using an electronic particle counter (Abbott Cell-Dyn 3700 SL; Abbott Laboratories Diagnostic Division, Abbott Park, IL). Ferritin and parathyroid

hormone levels were measured via chemiluminescence assays performed using an autoanalyzer (DPC Immulite 2000; Scientific Affairs, Biermann, Germany). Serum hs-CRP levels were estimated using an *appropriate* kit (Dade Behring GmbH, Marburg, Germany) as was serum CC (kit from Dade Behring), using a Dade Behring BN II Nephelometer.

We measured TT levels on an Elecsys model E170 immunoassay analyzer (Roche Diagnostics) using a commercially available kit.

Serum prohepcidin levels were evaluated using an enzyme-linked immunoassay kit (EIA-4644; DRG Diagnostics/ Instruments GmbH, Marburg**,** Germany). The detection limit

Table I: Demographics and clinical characteristics of the patients.

		Group1 (n=22) Hemodialysis (HD)	**Group2 (n=21) Peritoneal dialysis (PD)**	**Group3 (n=16) Control**
Demographic	Age	43 ± 11	53 ± 7††	48 ± 6
	Gender Male Female	 12 (%54) 10 (%46)	 10 (%48) 11 (%52)	 8 (%50) 8 (%50)
	Blood pressure (mmHg)	101.3 ± 19.3*	103.9 ± 18.5*	92.1 ± 8.9
	Body mass index (kg/ m^2)	23.5 ± 4.3	24.5± 2.4	24.5 ± 3.7
	Dialysis time (month)	96.7 ± 65†	56.4 ± 27	
	Dialysis Kt/V	1.6 ± 0.2	2.0 ± 0.25	
Haematologic	Hemoglobin (g/dL)	11.2 ± 1.2***	11.0 ± 1.6***	13.6 ± 1.3
	Hematocrit (%)	33.2 ± 4.1***	32.9 ± 5.2***	40.6 ± 3.3
	MCV	88.4 ± 7.0	89.7 ± 5.2*	83.8 ± 5.9
Analytical characteristics	Creatinine (mg/dL)	10.6 ± 1.7***	9.8 ± 3.1***	0.86 ± 0.16
	Calcium x Phosphorus	46.6 ± 2.35*	43.7 ± 13.3*	31.2 ± 2.74
	Total protein (g/dL)	6.9 ± 0.4	6.6 ± 0.6	7.2 ± 0.4
	Albumin (g/dL)	4.2 ± 0.2 ††	3.8 ± 0.6***	4.5 ± 0.4
	Total cholesterol(mg/dL)	177.9 ± 37.5	227.7 ± 68.3††	196.3 ± 28.2
	HDL cholesterol (mg/dL)	44.2 ± 11.0*	49.1 ± 12.0	53.9 ± 13.1
	Triglyceride (mg/dL)	194.2 ± 94.6	183.6 ± 96.7	133.8 ± 65.7
	LDL cholesterol (mg/dL)	99.4 ± 27.4	142.8 ± 53.9††	115.5 ± 25.2
	Parathormon (pg/mL)	316.6 ± 260	436.9± 399.3	
Cardiovascular risk markers	Hs CRP (mg/L)	0.93 ± 0.8*	0.68 ± 1.2	0.25 ± 0.3
	Troponin T (ng/ml)	0.022 ± 0.05	0.053 ± 0.05**	0.0 ± 0.0
	Cystatin C (mg/L)	6.36 ± 0.9***	6.1 ± 1.7***	0.71 ± 0.1
	Prohepcidin (ng/ml)	277.3± 49.4***	312.2± 127.9***	51.5 ± 7.9
Echocardiographic markers	LV mass (gr)	220.86 ± 97.3	218.6 ± 93.5	160.17± 46.66
	LV mass index (gr/m^2)	128.8 ± 46.4*	127.4 ± 49.7*	90.01 ± 21

Abbreviations: ***p* < .05 versus control. ****p* < .01 versus control.*****p* < .001 versus control. †*p* < .05 HD versus PD patients. ††*p* < .01 HD versus PD patients. †††*p* < .001 HD versus PD patients. **LV:** Left ventricule. **MCV:** Mean corpuscular volume, **HDL:** High density lipoprotein, **LDL:** Low density lipoprotein. Hs **CRP:** High sensitive C reactive protein.

Table II: Prohepcidin, biochemical and other cardiovascular risk factors.

		Correlation coefficient (r)	p
Demographics	Age	.114	ns
	Body mass index	-.258	ns
	Dialysis time	-.151	ns
	Blood pressure	.179	ns
Analytics	Creatinine	.764	<0.001
	Total cholesterol	.381	0.01
	HDL cholesterol	-.073	ns
	LDL cholesterol	.428**	0.003
	Triglyceride	.258	ns
	Parathyroid hormone	-.015	ns
	Calcium X Phosphorus	.266	ns
	Hematocrit	-.527	<0.001
	Total Protein	-.262	ns
	Albumin	-.308*	0.040
Cardiovascular risk factors	hs-CRP	.179	ns
	Troponin T	.632**	<0.001
	Cystatin C	.855**	<0.001
Echocardiography	LV mass index	.435	0.003

LV: Left ventricule. **HDL:** High density lipoprotein, **LDL:** Low density lipoprotein. **Hs CRP:** High sensitive C reactive protein.

Table III: Regression analysis with prohepcidin as an independent variable.

	Coefficient	p
Cystatin C	0.855	<0.001
Troponin T	0.138	0.002
Hs-CRP	0.124	0.417
LV mass index	0.435	0.003
Hemoglobin	0.046	0.633

LV: Left ventricle. **Hs CRP:** High sensitive C reactive protein

was 4 ng/mL. The intra- and inter-assay coefficients of variation were both <7%. All measurements were performed in duplicate, in random order. Laboratory staff members were blinded to the clinical features of the patients.

Dialysis adequacy was calculated as Kt/V (K = dialyzer clearance of urea, t = dialysis time, and V = volume distribution of urea).

Echocardiography

Standard echocardiographic evaluation, in the two-dimensional (2D) and M modes, was performed using 2D guidance, employing a Diasonic 800 Vingmed Sound device (Horten, Norway) fitted with a 3.5 MHz transducer. Parasternal, longitudinal, transverse, and subcostal apical two-chamber and four-chamber views were obtained, following the recommendations of the American Society of Echocardiography (16). We determined the telesystolic and telediastolic diameter of each left ventricle, and the thicknesses of the interventricular septum (IVS) and the posterior ventricular wall. The left ventricular mass (LV mass) was calculated using the following formula (17): MVI (g) = 0.8 x 1.04 [(DTDVI + SIV + PPVI)3–DTDVI3) + 0.6]. The LVMI was calculated by dividing the LV mass (18) by body height and was expressed in g/m^2 (19).

Data Analysis

Statistical analysis was performed using SPSS version 13.0. Normally distributed data were compared using the Independent Samples t-test, and the Mann-Whitney U-test was applied

when parameters were not normally distributed. Correlations among study variables were tested by calculation of Pearson's correlation coefficients. Categorical values were compared using Pearson's Chi-squared test and/or Fisher's exact test. A difference was considered to be statistically significant when $p<0.05$.

RESULTS

Demographic and laboratory data for all subjects are summarized in Table I. The levels of the CVD risk markers TT, CC, hemoglobin and prohepcidin differed, with statistical significance, between the patient and control groups. Additionally, both prohepcidin level and the LVMI were significantly different when control values were compared with those of hemodialysis and peritoneal dialysis patients.

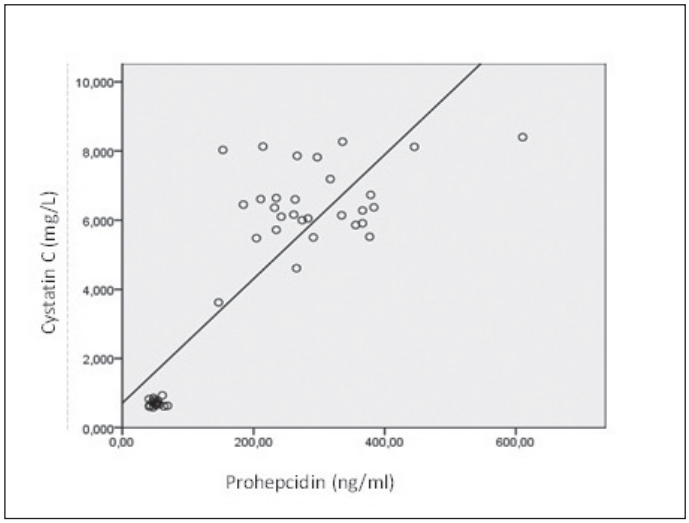

Figure 1: *Scattergram and regression line showing a significant relationship between prohepcidin and Cystatin C.*

The calculated correlations between different parameters are presented in Table II. Correlations were evident between prohepcidin level and the hemoglobin as well as the concentrations of serum creatinine, LDL cholesterol, albumin, CC, and TT. However, no significant correlation was observed when age, body mass index (BMI), duration of dialysis, MAP, and the levels of HDL-cholesterol, triglycerides, parathyroid hormone, total protein, and hs-CRP were compared (Table II).

Upon linear regression analysis, the prohepcidin level was found to be significantly associated with the CC concentration (Figure 2), and the LVMI (Figure 3) but not with hemoglobin or hs-CRP levels (Table III).

DISCUSSION

The hormone hepcidin, a 25-amino acid peptide, is the principal regulator of iron balance and recycling (5, 20). In addition, a growing body of evidence suggests that hepcidin may have a pro-atherogenic activity (9, 15). The critical roles played by hepcidin in inflammation and iron metabolism render the hormone a critical link between development of atherosclerosis and ESRD, leading to CVD (15). Many factors affect hepcidin levels. Anemia, hypoxia, and inflammation increase hormone production whereas administration of erythropoietin reduces circulating hepcidin levels (21).

In the present work, we observed higher levels of prohepcidin and other cardiovascular risk markers (CC, TT, hemoglobin and hs-CRP), and an elevated LVMI, in dialysis patients compared to controls. We also found that the prohepcidin level was somewhat higher in peritoneal dialysis than in hemodialysis patients, but this difference was not statistically significant. Consistently, the literature shows that serum prohepcidin, CC, TT, and hs-CRP levels are elevated and hemoglobin is decreased in patients undergoing chronic dialysis (22, 23).

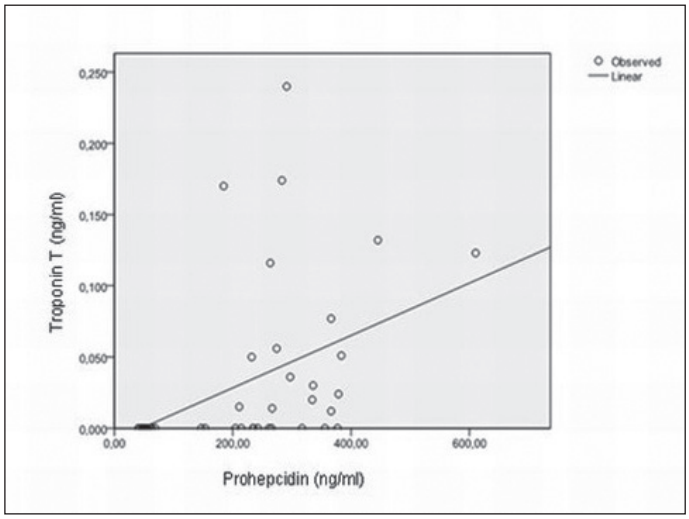

Figure 2: *Scattergram and regression line showing a significant relationship between prohepcidin and Troponin T.*

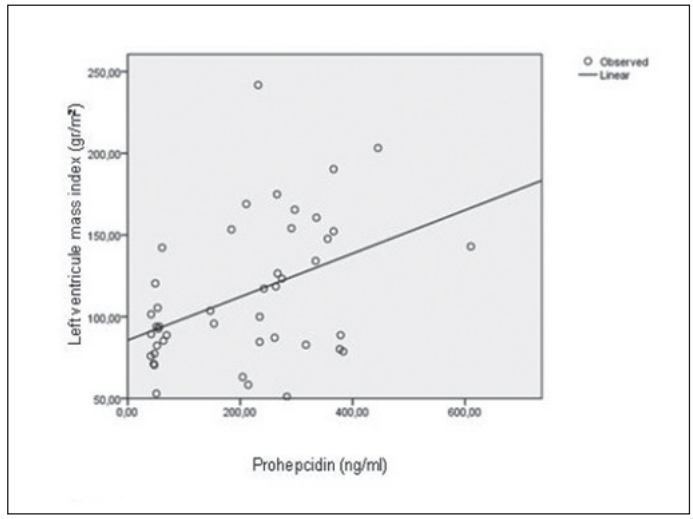

Figure 3: *Scattergram and regression line showing a significant relationship between prohepcidin and left ventricle mass index.*

Many studies have shown that hypoalbuminemia and anemia are strongly associated with mortality and CVD in dialysis patients (11, 23). As expected, a reduced hematocrit, and lower Hb and albumin levels were evident in our dialysis patients (compared to controls). Correlations were also evident between prohepcidin level and the hemoglobin and albumin concentration, in agreement with data of previous studies.

Recently, TT, CC, and CRP levels have been shown to be important markers of cardiovascular morbidity and mortality; such disease and death levels were elevated more than 7-fold in patients with higher levels of any of the three biomarkers and over 16-fold in patients with elevated levels of all markers (24). The principal finding of our current study was that prohepcidin levels in all patients correlated positively with the concentrations of the cardiovascular risk factors CC and TT, and the LVMI. Positive correlations between the levels of cystatin C, TT, and prohepcidin have previously been found in ESDR patients. However, to the best of our knowledge, we are the first to report the existence of a correlation between the LVMI and prohepcidin levels in ESDR patients. It is now well established that the LVMI is a powerful predictor of cardiovascular morbidity and mortality both in the general population and in patients with chronic kidney disease, including those on long-term dialysis (25, 26).

However, unlike what was found in previous studies, we failed to find any correlation between prohepcidin and hs-CRP levels in the ESRD group (Table II). This may be because chronic inflammation was well-controlled in our patients, or because of the low number of patients studied.

In conclusion, our data suggest that prohepcidin level, used as a biomarker of cardiovascular disease, is closely correlated with the cardiovascular risk indices of CC and TT concentrations, and the LVMI. In future, the use of all of these factors for prediction of death from cardiovascular causes will be diagnostically valuable, provided that our data are validated.

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