Serum Osteoprotegerin Levels Related With Cardiovascular Risk Factors in Chronic Kidney Disease

Pinar Demir,¹ Fusun Erdenen,^{2*} Hale Aral,³ Turker Emre,² Sennur Kose,²
Esma Altunoglu,² Anil Dolgun,⁴ Berrin Bercik Inal,³ and Aydin Turkmen⁵

¹Department of Internal Medicine, Ministry of Health Okmeydani Research and Training Hospital, Istanbul, Turkey

²Department of Internal Medicine, Ministry of Health Istanbul Research and Training Hospital, Istanbul, Turkey

³Department of Medical Biochemistry, Ministry of Health Istanbul Research and Training Hospital, Istanbul, Turkey

⁴Department of Biostatistics, Medical Faculty, Hacettepe University, Ankara, Turkey

⁵Department of Nephrology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

Background: To evaluate osteoprotegerin (OPG) levels in relation to cardiovascular (CV) risk factors in patients with chronic kidney disease (CKD) on different regimens of renal replacement therapy. **Methods:** A total of 143 patients with CKD and 30 healthy controls were included in this study and divided into five categories, including predialysis patients with chronic renal failure (preD; $n = 36$), chronic peritoneal dialysis patients (PD; $n = 36$), hemodialysis patients (HD; $n = 35$), renal transplant patients (RT; $n = 36$), and controls ($n = 30$). Data on demographics, concomitant diseases and CV risk factors, serum OPG levels, and correlates of serum OPG levels were determined. **Results:** Serum OPG (pmol/l) levels were significantly higher in HD ($P < 0.001$ for each), PD ($P < 0.001$ for each), and preD ($P < 0.01$ vs. control, $P < 0.05$ vs. RT) groups than RT and control groups.

Key words: chronic kidney disease; renal replacement therapy; osteoprotegerin; cardiovascular risk

Diabetics than nondiabetics in HD ($P = 0.008$), PD ($P = 0.024$), and RT ($P = 0.004$) groups and males than females in PD group ($P = 0.021$) had higher OPG levels. Serum OPG levels were associated positively with age in HD ($P < 0.001$), PD ($P = 0.001$), and in overall population ($P < 0.001$). **Conclusion:** Our findings revealed increased serum levels of OPG in dialysis and preD patients compared to RT and controls. In the patient groups receiving two dialysis treatment, the levels were worse, indicating a more pronounced vascular injury. Age, C-reactive protein (CRP), high-density lipoprotein cholesterol (HDL-C), and cystatin C (CysC) in CKD patients, CRP and PTH in the control subjects, and age and BMI in the overall population were the significant correlates of serum OPG levels. J. Clin. Lab. Anal. 30:811–817, 2016. © 2016 Wiley Periodicals, Inc.

INTRODUCTION

Chronic kidney disease (CKD) has been consistently reported to be associated with excess cardiovascular (CV) risk, while cardiovascular disease (CVD) has been considered as the leading cause of mortality in patients with CKD who require renal replacement therapy with dialysis or kidney transplantation (1–4).

Increased risk for CV disease in renal patients has been attributed to the risk factors, such as hypertension (HT), oxidative stress, proteinuria-albuminuria,

anemia, abnormal calcium-phosphate metabolism, malnutrition, chronic low-grade inflammation, and dyslipidemia (3–6). Notably, vascular calcifications have been

*Correspondence to: Fusun Erdenen, Department of Internal Medicine, Ministry of Health Istanbul Research and Training Hospital, Istanbul, Turkey. E-mail: fusunozerdenen@hotmail.com

Received 25 December 2014; Accepted 7 January 2016

DOI 10.1002/jcla.21941

Published online in Wiley Online Library (wileyonlinelibrary.com).

increasingly recognized among patients with CKD as an important risk factor for uremia-induced CV disease and an important predictor of all-cause as well as CV mortality (6, 7). Hence, in addition to traditional risk factors for atherosclerosis such as dyslipidemia, HT, smoking, and age, kidney-specific mechanisms including uremia, abnormalities in mineral metabolism, increased levels of potentiating molecules, decreased levels of inhibitory molecules, and calcium-phosphate flux related to dialysis have been suggested in the pathogenesis of vascular pathology (8–11). In this context being expressed and/or released from these cells, bone matrix proteins including osteoprotegerin (OPG), receptor activator of nuclear factor kappa B ligand (RANKL), and osteopontin (OP) have been considered to be regulating molecules and identified as biological risk factors for arteriosclerosis (12–15).

OPG, called “bone protector,” is a member of the tumor necrosis factor- α family that inhibits the maturation of osteoclast progenitor. Osteogenic markers including OPG are suggested to play an important role in mineralization of ectopic sites and are linked to vascular calcification. Although OPG is suggested to have an active role in vascular pathophysiology, it is not currently clear whether this role is beneficial or injurious to the vasculature. Anticalcification activity of OP and OPG was suggested to be a part of compensatory vascular defense system upregulated in atherosclerosis (4, 12, 16–18).

Hence limited data are available on serum OPG levels with respect to the different regimens of renal replacement therapy; the present study was designed to evaluate OPG levels in relation to CV risk factors in patients divided into four categories, including pre-dialysis (preD) patients with chronic renal failure, peritoneal dialysis (PD), hemodialysis (HD), and renal transplant (RT) patients.

METHODS

Study Population

A total of 143 patients with CKD and 30 healthy controls were included in this study conducted at two tertiary care centers. Patients were divided into four categories, including preD patients with chronic renal failure ($n = 36$), PD patients ($n = 36$), HD patients ($n = 35$), and RT patients ($n = 36$). Exclusion criteria included malignancy, clinical signs of acute infection, HIV infection, chronic inflammatory disease, pregnancy, breast feeding, and unwillingness to participate in the study. Thirty individuals with normal physical examination and laboratory results selected from subjects without any history of known chronic disease or drugs were taken as the control group.

Written informed consent was obtained from each subject following a detailed explanation of the objectives

and protocol of the study which was conducted in accordance with the ethical principles stated in the “Declaration of Helsinki” and approved by the institutional ethics committee.

Assessments

Data on demographic and clinical characteristics of patients, and comorbidities such as diabetes mellitus (DM), HT, ischemic heart disease (IHD) were retrieved from medical records in patient and control groups. Blood biochemistry findings related to CV risk factors and serum OPG levels were determined in each participant. Correlates of serum OPG levels were determined via linear regression analysis.

Dialysis Therapy

HD patients received dialysis three times weekly for at least 4 hr via native arteriovenous fistula for at least 6 months. Blood flow was standardized to 250–300 ml/min, and dialysate flow to 500 ml/min. PD patients received continuous ambulatory peritoneal dialysis (CAPD, $n = 24$) and automated peritoneal dialysis (APD, $n = 12$) treatments. All the patients had tunneled Tenckhoff catheter. Duration of dialysis for both PD and HD were detected in a range of 7 months to 15 years.

Laboratory Analysis

Venous blood collection was performed at the beginning of HD or PD. Serum samples for cystatin C (CysC) and OPG were frozen at -80°C . In routine practice, biochemical parameters including hemoglobin, high-density lipoprotein cholesterol (HDL-C), albumin, sodium, calcium (Ca), inorganic phosphorous (iP), parathormone (PTH), C-reactive protein (CRP), and CysC were measured in the medical biochemistry laboratory (Siemens Healthcare Diagnostics, Terrytown, New York). Low-density lipoprotein cholesterol (LDL-C) levels were estimated by using Friedewald formula. Unfortunately, we could not collect blood sample enough for serum CRP measurements of the preD group ($n = 2$).

When serum containing CysC is mixed with the latex particles coated with rabbit anti-CysC antibody, agglutination takes place, resulting in an increase in turbidity. According to the kit insert, the intra- and interassay precisions coefficients of variation (CV) were 1.0% and 1.8% at a low level of 0.60 mg/l, and 1.8% and 3.1% at a high level of 4.95 mg/l, respectively.

Method of enzyme-linked immunosorbent assay (ELISA) was used in determination of serum OPG levels (catalog no.: BI-20402, Biomedica, Germany). Microwells were coated with mouse anti-OPG, and sandwich

technique was used with polyclonal anti-OPG antibodies sourced from goats. According to the kit insert, the median was found as 1.8 pmol/l ($N = 1,134$). The intraassay precisions CV were 10% at a low level of 4.59 pmol/l, and 4% at a high level of 10.76 pmol/l. The interassay precisions CV were 7% at a low level of 5.53 pmol/l, and 8% at a high level of 10.1 pmol/l.

Statistical Analysis

Statistical analysis was made using computer software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Kolmogorov–Smirnov test was used to check the normality of data among groups. ANOVA and post hoc least significant difference (LSD) tests were used for comparison of normally distributed data, while Kruskal–Wallis and post hoc Conover tests were used for nonnormally distributed data. The potential correlations between OPG and biochemical parameters were investigated with univariate Pearson's and Spearman's correlation analysis. To investigate the factors influencing OPG, multiple linear regression analysis was conducted by using the "enter method." After adjusting for age serum OPG levels between groups were compared via covariance analysis. Data were expressed as "mean (standard deviation; SD)", minimum–maximum, and percent (%) where appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics in Study Groups

Patient and control groups were similar in terms of gender, while patients in HD, PD, and preD groups were older compared to control and RT groups ($P < 0.001$ for each), and BMI (kg/m^2) values in the HD and PD groups were significantly lower than in preD and control groups ($P < 0.005$ for each; Table 1).

Blood Biochemistry in Study Groups

Significantly lower levels for hemoglobin, albumin, and HDL-C, and higher levels for $\text{Ca} \times \text{iP}$ values were determined in HD, PD, and preD groups compared with RT group and control subjects (P values ranged from <0.005 to <0.001). Highest level of serum sodium (Na) were noted among patients in RT group ($P < 0.001$), while highest levels for serum PTH in PD group ($P < 0.001$ for each). CRP (mg/l) levels in HD ($P < 0.001$) and PD ($P < 0.05$) groups, while CysC (mg/dl) in all patient groups were significantly higher than in control subjects ($P < 0.001$ for HD, PD, and preD groups, $P < 0.05$ for RT group; Table 1).

OPG Levels in Study Groups With Respect to Comorbid Disorders

Serum OPG (pmol/l) levels were significantly higher in HD ($P < 0.001$ for each), PD ($P < 0.001$ for each), and preD ($P < 0.01$ vs. control, $P < 0.05$ vs. RT) groups (Table 1). Higher OPG levels were detected in patients with DM than nondiabetics in HD ($P = 0.008$), PD ($P = 0.024$), and RT ($P = 0.004$) groups. In patients with coronary heart disease (CHD), OPG levels were significantly higher compared to patients without CHD in HD ($P < 0.001$) group (Table 2).

Correlation of Serum OPG Levels With BMI and Lipid Parameters

OPG levels were correlated positively with age in HD ($r = 0.664$, $P = 0.0001$) and PD ($r = 0.518$, $P = 0.001$) groups, with HDL-C in HD ($r = 0.335$, $P = 0.049$) and preD ($r = 0.369$, $P = 0.027$) groups, while negatively with total cholesterol ($r = -0.334$, $P = 0.047$) and LDL-C ($r = -0.352$, $P = 0.036$) in RT group. However, OPG levels were correlated negatively with BMI ($P = 0.013$) in the overall population.

Multivariate Regression Analysis for Correlates of Serum OPG Levels

Linear regression analysis revealed that serum levels for OPG were likely to increase by 0.29 unit for each 1 year increase in age ($P = 0.001$) in HD group; by 0.26 unit for each 1 year increase in age ($P = 0.001$) and by 0.95 unit for each 1 unit increase in CRP ($P = 0.030$) in PD group; by 0.15 unit for each 1 unit increase in HDL-C ($P = 0.006$), by 0.55 unit for each 1 unit increase in sodium ($P = 0.029$), and by 2.16 unit for each 1 unit increase in CysC ($P = 0.007$) in preD group. In the control group, serum levels for OPG were likely to increase by 3.21 unit for each 1 unit increase in CRP ($P = 0.003$) and by 0.01 unit for each 1 unit increase in PTH ($P = 0.022$). In the overall study population, serum levels for OPG were likely to increase by 0.30 unit for each 1 year increase in age ($P < 0.001$) and to decrease by 0.22 unit for each 1 unit increase in BMI ($P = 0.013$; Table 3).

DISCUSSION

To our knowledge there is limited data on comparison of different renal replacement therapies with respect to OPG. In some studies with stage 5 CKD patients (prior to renal replacement therapy), higher levels of OPG were associated positively with older age, male gender, high CRP and HbA1c values, protein-energy wasting, and carotid plaques; and negatively with albumin and hemoglobin levels (19), and coronary artery calcification (20).

TABLE 1. Comparison of Demographic Characteristics and Laboratory Findings in Study Groups

	HD (<i>n</i> = 35)	PD (<i>n</i> = 36)	preD (<i>n</i> = 36)	RT (<i>n</i> = 36)	Control (<i>n</i> = 30)	<i>P</i> -value
Age (years)	52.0 (15.5) ^{***,+++}	51.2 (9.8) ^{***,+++}	54.6 (9.4) ^{***,+++}	40.1 (13.1)	36.5 (11.4)	<0.001
Males (%)	51.4	50	50	36.1	33.3	0.392
Smoking (%)	42.9 ^{**} , ⁺⁺ , ^{qq} , ^{ww}	25.0	25.0	0.0 ^{**} , ^{qq} , ^{ww}	13.3	<0.001
DM (%)	31.4	30.6	47.2	25		0.220
IHD (%)	22.9	22.2	13.9	5.6		0.152
HT (%)	24.4	22.7	28.6	24.4		0.159
BMI (kg/m ²)	24.1 (5.0) [*] , ⁺ , ^{qq}	25.0 (4.2) [*] , ^q	27.3 (4.9)	26.5 (5.8)	27.4 (5.6)	0.025
SBP (mmHg)	122.3 (26.4) ^{qqq}	131.7 (24.5) ^q	142.5 (23.5) ^{***}	128.3 (18.3) ^{qq}	118.3 (9.1)	<0.001
Duration of therapy (months)	47.7 (26.4) ^{qqq}	60.3 (37.5) ^{qqq}	4.5 (0.6)			<0.001
Hb (g/dl)	10.8 (1.4) ^{***,+++}	11.2 (1.8) ^{***,+++}	11.1 (1.7) ^{***,+++}	13.0 (1.5)	13.7 (1.5)	<0.001
Glukoz (mg/dl)	1251 (70.9)	123.1 (63.8)	115.0 (45.6)	101.3 (38.9)	91.7 (7.0)	0.138
Alb (g/dl)	4.0 (0.4) ^{***,+++} , ^{qqq}	3.7 (0.4) ^{***,+++} , ^{qqq}	4.1 (0.4) ^{***,+++}	4.6 (0.3)	4.5 (0.2)	<0.001
HDL-C (mg/dl)	34.3 (10.8) ^{***,+++} , ^{qq}	37.9 (9.7) ^{***,+++}	40.7 (9.4) [*] , ⁺⁺⁺	55.0 (12.7) ^{**}	49.1 (12.1)	<0.001
Ca × iP	48.7 (11.3) ^{***,+++} , ^{qq}	48.8 (16.9) ^{***,+++} , ^{qqq}	39.9 (8.3) [*] , ⁺⁺⁺	30.0 (5.6)	34.1 (6.3)	<0.001
Na (mmol/l)	137.0 (3.1) ^{***,+++} , ^{qqq} , ^{www}	139.7 (2.9)	140.4 (2.1)	141.8 (2.2) [*] , ^q , ^{www}	140.6 (1.8)	<0.001
CRP (mg/l)	2.0 (2.7) ^{***}	1.3 (1.8) [*]		1.6 (1.4)	0.3 (0.1)	0.002
PTH (pg/ml)	573.9 (798.2) ^{**} , ⁺⁺ , ^{www}	1029.5 (973.8) ^{***,+++} , ^{qqq}	339.9 (206.1)	106.2 (77.7)	65.1 (32.1)	<0.001
OPG (pmol/l)	Mean(SD) 14.0 (7.7) ^{***,+++} , ^{qqq} , ^w	11.5 (5.7) ^{***,+++} , ^{qqq}	6.8 (3.4) ^{**} , ⁺	4.5 (2.1)	2.7 (1.0)	<0.001
	Median 12.7	10.4	6.6	4.0	2.8	
CysC (mg/dl)	6.2 (1.4) ^{***,+++} , ^{qqq}	5.9 (1.1) ^{***,+++} , ^{qqq}	3.4 (0.7) ^{***,+++}	1.3 (0.3) ^{**}	0.7 (0.1)	<0.001

P* < 0.05.*P* < 0.01.****P* < 0.001.

Compared to control group.

+*P* < 0.05.++*P* < 0.01.+++*P* < 0.001.

Compared to RT group.

q*P* < 0.05.qq*P* < 0.01.qqq*P* < 0.001.

Compared to preD group.

w*P* < 0.05.ww*P* < 0.01.www*P* < 0.001.

Compared to PD group. Data are shown as mean (SD).

Similarly, age, CRP, HDL-C and CysC in our patients, CRP and PTH in the control group and age and BMI in the overall population were the significant correlates of serum OPG levels in the present study (Table 3). Multivariate analysis revealed a correlation between BMI

and OPG in the whole group (*P* = 0.013). Additionally, consistent with identification of highest levels for OPG in our HD group patients, HD patients were reported to show a higher progression of coronary artery calcification scores associated with age, BMI, OPG, and Framingham

TABLE 2. Serum Levels for OPG With Respect to Concomitant DM and Coronary Heart Disease in Study Groups

	Serum OPG levels (pmol/l)					
	DM (−)	DM (+)	<i>P</i> -value	CHD (−)	CHD (+)	<i>P</i> -value
HD	11.8 (7.0)	18.9 (7.0)	0.008	11.6 (5.4)	22.3 (8.7)	0.0001
PD	10.1 (5.1)	14.7 (5.9)	0.024	11.3 (6.0)	12.1 (5.0)	0.757
Predialysis	6.0 (2.4)	7.6 (4.2)	0.181	6.6 (3.4)	7.6 (3.8)	0.569
Renal transplantation	4.0 (1.9)	6.2 (1.9)	0.004	4.4 (2.0)	7.1 (1.1)	0.079

DM, diabetes mellitus; CHD, coronary heart disease.

TABLE 3. Multivariate Linear Regression Analysis for Correlates of Serum OPG Levels

Group	Variable	Beta	SE	P-value
HD	Constant	-4.59	14.67	0.756
	Age (years)	0.29	0.07	<0.001
	Albumin (g/dl)	-0.47	3.26	0.886
	Therapy (month)	0.02	0.02	0.408
	HDL-C (mg/dl)	0.11	0.09	0.253
PD	Constant	7.17	11.07	0.521
	Age (years)	0.26	0.07	0.001
	Albumin (g/dl)	-3.22	2.29	0.170
	CRP (mg/l)	0.95	0.42	0.030
	Therapy (month)	0.02	0.02	0.271
PreD	Constant	-84.11	34.83	0.021
	HDL-C (mg/dl)	0.15	0.05	0.006
	Sodium (mmol/l)	0.55	0.24	0.029
	CysC (mg/dl)	2.16	0.75	0.007
Control	Constant	1.22	0.39	0.004
	CRP (mg/l)	3.21	1.01	0.003
	PTH (pg/ml)	0.01	0.001	0.022
	CysC (mg/dl)	1.22	0.63	0.059
Overall	Constant	35.81	29.36	0.227
	Age (years)	0.30	0.06	<0.001
	BMI (kg/m ²)	-0.22	0.08	0.013
	Therapy (month)	0.02	0.02	0.358
	Hemoglobin (g/dl)	-0.06	0.47	0.891
	Albumin (g/dl)	-1.62	2.29	0.481
	HDL-C (mg/dl)	0.10	0.07	0.154
	Ca × iP	-0.01	0.05	0.727
	Sodium (mmol/l)	-0.31	0.21	0.150
	CRP (mg/l)	0.25	0.32	0.439
	PTH (pg/ml)	-0.001	0.001	0.513

There was no valid linear regression model for the renal transplanted group.

risk index (6). Higher OPG levels were reported in pediatric HD than PD patients (21).

Significantly higher values for serum OPG were noted in our dialysis (HD and PD) groups than preD, RT, and control groups consistent with literature in CKD patients (21–23). Besides, identification of lowest serum levels of OPG in the RT group supports the data on the reduction of previously increased serum OPG levels in uremic patients after the transplantation (7, 23–25).

After adjusting the effects of other factors by using multivariate regression analysis, a positive correlation between age and OPG was shown in dialysis groups and in the overall study population in the present study, which supports findings of past studies (17, 18, 26–28). We found age and OPG was correlated in HD, PD groups, and in the whole group (all *P* values were **<0.001**). Conflicting results of OPG levels were reported to be associated with smoking (17, 26, 29, 30) and duration of dialysis therapy (5, 7, 24). Neither smoking nor duration of dialysis was among the correlates of OPG levels in our study population, while alcohol consumption could not be analyzed

Fig. 1. Dot plot showing the distribution of serum OPG levels among groups after adjusted with age. In the plot, dots represent the adjusted values of serum OPG levels and the horizontal lines represent the median of OPG levels for each group.

due to very low rates of alcohol use (in three patients overall).

Several recent reports have indicated that CysC may be a better predictor of adverse CV events and all-cause mortality than either serum creatinine or creatinine-based estimating equations (4, 31) in CKD. In our study population, CysC levels were among the significant correlates of serum OPG levels in preD group and showed tendency for a significant relation in the overall study population. Elevated OPG levels were associated with long-term renal dysfunction in older women in a study using serum creatinine and CysC-based estimated glomerular filtration rate (32).

Data on the association between OPG and CRP levels revealed a strong correlation between two parameters (17, 19, 28, 30). CRP levels were found higher than controls both in HD and PD patients (33, 34). Although preD, PD, and HD patients had higher OPG, and dialysis patients had higher CRP levels in our research, a correlation with CRP and OPG was observed in PD group (*P* = 0.030), and in control group (*P* = 0.003) by multivariate analysis. This may be related to several confounding factors likely to affect CRP and OPG levels. Higher levels for serum CRP in HD compared with PD group in our study population seems to emphasize previously suggested role of extracorporeal circulation of blood during HD, which acts as a repeated stimulus for an inflammatory response and the positive association of higher plasma urea levels and CRP (35). With respect to higher OPG levels in HD patients than PD patients, this difference disappeared after adjusting with age, but remained still higher than controls and RT patients (Fig. 1). Klejna

et al. reported that OPG is not removed through the polysulfon membranes (36); and the monomers are not eliminated through glomerular membrane filtration either. Although the exact mechanism is not clear, this may be due to the kidneys' role in OPG metabolism and clearance (37). Increased levels of OPG in dialysis patients may be related to uremic stimulation and the dialysis procedure itself (36).

Although higher OPG levels were detected in patients with DM than nondiabetics in our HD, PD, and RT groups, there was no significant difference in the PreD group (Table 2). Elevated OPG levels were associated with the endothelial dysfunction determined by a decreased flow-mediated dilatation of the brachial artery in diabetics (38). On the other hand, in end stage renal disease, higher levels of OPG were associated with significantly greater risk of all-cause and CV mortality among nondiabetics, independent of inflammation. Diabetes is a strong independent stimulus for vascular calcification and is associated with poor outcomes among patients receiving dialysis. It is possible that among a population with a very high prevalence of vascular calcification and poor outcomes, higher levels of calcification biomarkers may be less informative (39).

In our study group, the critical value for $\text{Ca} \times \text{iP}$ (37.535) by using receiver operating characteristics was very similar to the median value of the whole group (37.730), although it showed weak diagnostic performance area under curve ($\text{AUC} = 0.678$) (40). The recommended target of $<55 \text{ mg}^2 / \text{dl}^2$ (41) should be revised to predict the extraskeletal calcification threatening the lifespan of the patient with CKD. Serum levels of OPG was reported to be independent from concomitant DM, serum levels of PTH, Ca, iP, cholesterol, triglyceride, or use of phosphate binders or vitamin D, and not correlated to Ca, iP, $\text{Ca} \times \text{iP}$ product and PTH (7).

As our groups are small and heterogeneous, some limitations to this study should be considered. The first limitation is the cross-sectional design making impossible to establish any cause and effect relationships in such a short period of time. Second, neither the etiology of renal disease in the patient groups, nor the metabolic bone diseases which may have influenced serum OPG levels were documented. Third, we may have underestimated the true prevalence of vascular disease as our diagnoses were based on the clinical history; our research does not include any evaluation of vascular pathology along with assessment of life-style characteristics, oxidative stress markers, bone turnover markers, and endothelial or CV status. Although we could not demonstrate the clinical outcomes according to OPG levels in different groups either, this is a preliminary study and we are gathering information of our patients to see the association of this biomarker with CV progress and/or death.

CONCLUSION

In the present study, we found that serum OPG levels were increased in CKD patients with respect to different replacement therapies. In the patients receiving two dialysis treatments and preD patients, who were also elder, the levels were worse, which may result in more pronounced vascular outcomes. Lower OPG levels in RT group (being slightly higher than control subjects but significantly lower than PD, HD, and preD patient groups) suggested us, along with other advantages, transplantation is the best renal replacement therapy by changing most of the injurious stimuli including OPG, and milieu in CKD patients.

REFERENCES

1. Moody WE, Edwards NC, Madhani M, et al. Endothelial dysfunction and cardiovascular disease in early-stage chronic kidney disease: Cause or association? *Atherosclerosis* 2012;223:86–94.
2. Szeto CC, Wong TY, Chow KM, Leung CB, Li PK. Are peritoneal dialysis patients with and without residual renal function equivalent for survival study? Insight from a retrospective review of the cause of death. *Nephrol Dial Transplant* 2003;18:977–982.
3. Sarnak MJ. Cardiovascular complications in chronic kidney disease. *Am J Kidney Dis* 2003;41(Suppl 5):S11–S17.
4. Schiffrin EL, Lipman ML, Mann JF. Chronic kidney disease: Effects on the cardiovascular system. *Circulation* 2007;116:85–97.
5. Hujairi NMA, Afzali B, Goldsmith DJA. Cardiac calcification in renal patients: What we do and don't know. *Am J Kidney Dis* 2004;43:234–243.
6. Barreto DV, Barreto FC, Carvalho AB, et al. Coronary calcification in hemodialysis patients: The contribution of traditional and uremia-related risk factors. *Kidney Int* 2005;67:1576–1582.
7. Moe SM, Reslerova M, Ketteler M, et al. Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). *Kidney Int* 2005;67:2295–2304.
8. Huang JW, Lien YC, Yang CY, et al. Osteoprotegerin, inflammation and dyslipidemia are associated with abdominal aortic calcification in non-diabetic patients on peritoneal dialysis. *Nutr Metab Cardiovasc Dis* 2014;24:236–242.
9. Dellegrottaglie S, Sanz J, Rajagopalan S. Vascular calcification in patients with chronic kidney disease. *Blood Purif* 2006;24:56–62.
10. Goodman WG, Goldin J, Kuizon BD, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 2000;342:1478–1483.
11. Giachelli CM. Mechanisms of vascular calcification in uremia. *Semin Nephrol* 2004;24:401–402.
12. Schoppet M, Shroff RC, Hofbauer LC, Shanahan CM. Exploring the biology of vascular calcification in chronic kidney disease: What's circulating? *Kidney Int* 2008;73:384–390.
13. Kaden JJ, Bickelhaupt S, Grobholz R, et al. Receptor activator of nuclear factor kappa B ligand and osteoprotegerin regulate aortic valve calcification. *J Mol Cell Cardiol* 2004;36:57–66.
14. Nitta K, Akiba T, Uchida K, et al. Serum osteoprotegerin levels and the extent of vascular calcification in haemodialysis patients. *Nephrol Dial Transplant* 2004;19:1886–1889.
15. Tousoulis D, Siasos G, Maniatis K, et al. Serum osteoprotegerin and osteopontin levels are associated with arterial stiffness and the presence and severity of coronary artery disease. *Int J Cardiol* 2013;167:1924–1928.

16. Schoppet M, Preissner KT, Hofbauer LC. RANK ligand and osteoprotegerin paracrine regulators of bone metabolism and vascular function. *Arterioscler Thromb Vasc Biol* 2002;22:549–553.
17. Kiechl S, Schett G, Wenning G, et al. Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. *Circulation* 2004;109:2175–2180.
18. Ramirez-Sandoval JC, Casanova I, Villar-Tapia JA, et al. Biomarkers associated with vascular calcification in peritoneal dialysis. *Perit Dial Int*. 2015 Aug 20. pii: pdi.2014.00250. [Epub ahead of print].
19. Matsubara K, Stenvinkel P, Qureshi AR, et al. Inflammation modifies the association of osteoprotegerin with mortality in chronic kidney disease. *J Nephrol* 2009;22:774–783.
20. Morena M, Jaussent I, Dupuy AM, et al. Osteoprotegerin and sclerostin in chronic kidney disease prior to dialysis: Potential partners in vascular calcifications. *Nephrol Dial Transplant* 2015;30:1345–1356.
21. Shroff RC, Shah V, Hiorns MP, et al. The circulating calcification inhibitors, fetuin-A and osteoprotegerin, but not matrix Gla protein, are associated with vascular stiffness and calcification in children on dialysis. *Nephrol Dial Transplant* 2008;23:3263–3271.
22. Jiang JQ, Lin S, Xu PC, Zheng ZF, Jia JY. Serum osteoprotegerin measurement for early diagnosis of chronic kidney disease-mineral and bone disorder. *Nephrology (Carlton)* 2011;16:588–594.
23. Bargnoux AS, Dupuy AM, Garrigue V, Deleuze S, Cristol JP, Mourad G. Renal transplantation decreases osteoprotegerin levels. *Transplant Proc*. 2006;38:2317–2318.
24. Klejna K, Naumnik B, Gasowska K, Myśliwiec M. OPG/RANK/RANKL signaling system and its significance in nephrology. *Folia Histochem Cytobiol* 2009;47:199–206.
25. Sato T, Tominaga Y, Iwasaki Y et al. Osteoprotegerin level before and after renal transplantation. *Am J Kidney Dis*. 2001;38(Suppl 1):175–177.
26. Kadoglou NPE, Gerasimidis T, Golemati S, Kapelouzou A, Karayannacos PE. The relationship between serum levels of vascular calcification inhibitors and carotid plaque vulnerability. *J Vasc Surg* 2008;47:55–62.
27. Jono S, Ikari Y, Shioi A, et al. Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. *Circulation* 2002;106:1192–1194.
28. Stepien E, Wypasek E, Stopyra K, et al. Increased levels of bone remodeling biomarkers (osteoprotegerin and osteopontin) in hypertensive individuals. *Clin Biochem* 2011;44:826–831.
29. Browner WS, Lui L, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. *J Clin Endocrinol Metab* 2001;86:631–637.
30. Rhee EJ, Lee WY, Kim SY, et al. Relationship of serum osteoprotegerin levels with coronary artery disease severity, left ventricular hypertrophy and C-reactive protein. *Clin Sci* 2005;108:237–243.
31. Astor BC, Shafi T, Hoogeveen RC, et al. Novel markers of kidney function as predictors of ESRD, cardiovascular disease, and mortality in the general population. *Am J Kidney Dis* 2012;59:653–662.
32. Lewis JR, Lim WH, Zhu K, et al. Elevated osteoprotegerin predicts declining renal function in elderly women: A 10-year prospective cohort study. *Am J Nephrol* 2014;39:66–74.
33. Pencak P, Czerwieńska B, Ficek R, et al. Calcification of coronary arteries and abdominal aorta in relation to traditional and novel risk factors of atherosclerosis in hemodialysis patients. *BMC Nephrol* 2013 Jan 14;14:10. doi: 10.1186/1471-2369-14-10.
34. Lavín-Gómez BA, Palomar-Fontanet R, Gago-Fraile M, et al. Inflammation markers, chronic kidney disease, and renal replacement therapy. *Adv Perit Dial* 2011;27:33–37.
35. Uzun H, Konukoglu D, Besler M, Erdenen F, Sezgin C, Muderrisoglu C. The effects of renal replacement therapy on plasma asymmetric dimethylarginine, nitric oxide and C-reactive protein levels. *Clin Invest Med* 2008;31:E1–E7.
36. Klejna K, Naumnik B, Gasowska K, Myśliwiec M. OPG/RANK/RANKL signaling system and its significance in nephrology. *Folia Histochem Cytobiol* 2009;47:199–206.
37. Svensson M, Dahle DO, Mjølén G, et al. Osteoprotegerin as a predictor of renal and cardiovascular outcomes in renal transplant recipients: Follow-up data from the ALERT study. *Nephrol Dial Transplant* 2012;27:2571–2575.
38. Morisawa T, Nakagomi A, Kohashi K, et al. Osteoprotegerin is associated with endothelial function and predicts early carotid atherosclerosis in patients with coronary artery disease. *In Heart J* 2015;56:605–612.
39. Scialla JJ, Kao WH, Crainiceanu C, et al. Biomarkers of vascular calcification and mortality in patients with ESRD. *Clin J Am Soc Nephrol* 2014;9:745–755.
40. Aral H, Kural A, Demir P, et al. The importance of calcium phosphorus product in renal insufficiency. *Eur J Basic Med Sci* 2014;4:1–6.
41. Cicekcioglu H, Ergun I, Ucar O, et al. Cardiac complications of secondary hyperparathyroidism in chronic hemodialysis patients. *Turk J Med Sci* 2011;41:789–794.