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ORIGINAL ARTICLE

Plasma osteoprotegerin, arterial stiffness, and mortality in normoalbuminemic Japanese hemodialysis patients

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Abstract
Summary A high circulating osteoprotegerin (OPG) level may be a risk factor for vascular calcification and mortality in hemodialysis patients. OPG and pulse wave velocity (PWV) were measured at baseline in 151 normoalbuminemic, long-term (>3 years) Japanese hemodialysis patients who were prospectively followed for 6 years. In long-term normoalbuminemic Japanese hemodialysis patients, OPG levels were strongly linked with both arterial stiffness and worse outcome.
Introduction A high circulating OPG level is reported to be a risk factor for vascular calcification and mortality in Western chronic kidney disease (CKD) patients but it is not known if this is true for Japanese CKD patients, where a different risk profile may operate.
Methods OPG and PWV were measured at baseline in 151 normoalbuminemic, long-term (>3 years) Japanese hemodialysis patients (median age 62 years) who were prospectively followed for 6 years.
Results OPG levels were associated in multivariate analysis with age, dialysis vintage, history of cardiovascular disease (CVD) and parathyroid hormone levels. C-reactive protein levels did not correlate with OPG. Patients with clinical history of CVD had significantly higher OPG levels and OPG levels were positively correlated to PWV, an index of arterial stiffness. These associations were independent of age, sex, dialysis vintage, and diabetes. During the follow-up period, 40 deaths, including 25 cardiovascular deaths, were recorded. In crude analysis, each unit of increase in OPG was associated with increased all-cause (hazard ratios 1.14, 95% confidence interval 1.08–1.20) and CVD mortality (1.14 [1.07–1.21]), which persisted after adjustment for age, sex, dialysis vintage, diabetes, and baseline CVD (1.12 [1.05–1.19] and 1.11 [1.02–1.19], all-cause and CVD mortality, respectively).
Conclusions In long-term normoalbuminemic Japanese hemodialysis patients, with low prevalence of inflammation, OPG levels were strongly linked with both arterial stiffness and worse outcome.

Keywords Cardiovascular disease (CVD) · Hemodialysis (HD) · Mortality · Osteoprotegerin (OPG) · Pulse wave velocity (PWV)

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Introduction

Patients with chronic kidney disease (CKD) have a high risk of dying prematurely due to cardiovascular disease (CVD)

and one strong contributing factor is vascular calcification resulting in arterial stiffness [1]. Vascular calcification in CKD patients may be caused by underlying conditions (such as diabetes mellitus, atherosclerosis, inflammation, and certain medications) as well as by uremic disorders in bone and mineral metabolism including those of calcium, phosphate, parathyroid hormone (PTH), vitamin D, and proteins related to bone turnover and vascular calcification, such as osteoprotegerin (OPG) [1]. Pulse wave velocity (PWV) is often used as a surrogate indicator of arterial stiffness in CKD patients [2], and is a strong predictor of CVD mortality in hemodialysis (HD) patients [3].

Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor family and a decoy receptor that blocks the interaction between the receptor activator of nuclear factor-κB with its ligand, thus inhibiting osteoclast differentiation and activity [4, 5]. On the other hand, OPG knockout mouse exhibited an increase of arterial calcification [6, 7] which was prevented by experimentally obtained increased expression of OPG [7]. Despite this apparent protective effect on the vascular system and bone, the increased levels of circulating OPG levels in CKD patients [8] are reported to be associated with both aortic calcification [9, 10] and increased mortality [11, 12].

Although arterial calcification is highly prevalent in Japanese dialysis patients [13], mortality rates are much lower than in Western dialysis patients [14] suggesting that mortality risk factors may differ between these populations perhaps due to differences in genetic factors, nutrition, and life-style. OPG has been identified as a mortality risk factor in Western HD patients [11, 12], but this association is modified by the presence of concurrent inflammation [11, 15]. At present, it is unknown whether this is the case in Japanese patients as well, in whom, inflammation is a less common finding [16].

The aim of the present study was to look into the associations between OPG levels, arterial stiffness, and mortality in a cohort of long-term HD patients with normal albumin levels.

Subjects and methods

Study patients

This cross-sectional study with mortality follow-up including 151 ethnically Japanese HD patients from the Hakuai Hospital (currently: Hakuai Clinic, Kure, Japan). Inclusion criteria were long-term HD patients (over 3 years on HD) [8], normal albumin levels (serum albumin level≥35 g/L) [17], and not receiving intravenous active vitamin D therapy [18]. There were no patients who had evidence of acute infection at time of inclusion and none of the patients had undergone continuous ambulatory peritoneal dialysis or renal transplantation. Study entry and baseline sampling occurred in 1st July 2003. In a next step, patients were prospectively followed-up for mortality assessment until 30th July 2009. Cardiovascular mortality was defined as death resulting from coronary heart disease or congestive heart failure or stroke. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the hospital ethics committee. Signed informed consent was obtained from all patients before inclusion in this study.

Forty-three (29%) of the 151 patients presented diabetes mellitus at baseline, whereas 50 (33%) had a clinical history of CVD. Patients were treated with HD three times a week (4 h per session) using bicarbonate dialysate and high-flux dialysis membranes.

Biochemical parameters

Venous blood samples were collected before the HD session for routine measurement of serum concentrations of albumin (bromocresol purple), total cholesterol, high-density lipoprotein cholesterol (HDL cholesterol), calcium, phosphate, and C-reactive protein (CRP). The intact-parathyroid hormone (intact-PTH) was measured with a commercial kit from Roche Diagnostics (Tokyo, Japan). OPG was measured in duplicates using a sandwich enzyme immunoassay from Immundiagnostik (Bensheim, Germany) that employed two highly specific antibodies for human OPG. The adjusted calcium levels were calculated using Payne's formula [19] and the low-density lipoprotein cholesterol (LDL cholesterol) level was calculated using Friedewald's formula [20].

Brachial–ankle pulse wave velocity and ankle–brachial pressure index

Before HD, after resting for at least 10 min, PWV and ankle–brachial pressure index (ABI) was measured as previously described [21, 22] using a Vasera VS-1000 (Fukuda Denshi, Tokyo, Japan) which simultaneously recorded the PWV, ABI, blood pressure, electrocardiogram, and heart sounds. Normal ABI is defined as >0.9 [21]. An ABI <0.9 suggests the presence of arteriosclerosis obliterans, and in patients with such values the PWV measurement is underestimated.

Statistical analysis

All variables were expressed as the mean±SD or median (25th and 75th percentile), unless otherwise indicated. Statistical significance was set at the level of $P<0.05$. We used non-parametric Spearman's rank correlation analysis to determine the degree of association between OPG and other variables. Multivariate regression linear and logistic analyses were used

Table 1 General characteristics and univariate associations with plasma osteoprotegerin concentration in 151 hemodialysis patients

Parameter	All patients	Spearman's ρ	P value
OPG, pmol/L	10.5 (7.3 to 15.1)	–	–
Age, years	62.1 (±13.4)	0.35	<0.0001
Sex, male/female	85/66	0.05	0.52
Dialysis vintage, months	84 (48 to 132)	0.33	<0.0001
Diabetes mellitus,%	28.5	0.09	0.26
Clinical history of CVD,%	33.1	0.28	0.0006
Smoking,%	27.2	−0.13	0.12
Oral active vitamin D therapy,%	34.4	−0.11	0.21
Body mass index, kg/m^2	21.1 (±3.0)	−0.17	0.038
Systolic blood pressure, mmHg	149 (131 to 163)	0.06	0.48
Diastolic blood pressure, mmHg	88 (80 to 100)	−0.14	0.080
Albumin, g/L	40 (±3)	−0.19	0.020
Total cholesterol, mmol/L	4.4 (±0.9)	−0.17	0.038
HDL cholesterol, mmol/L	1.2 (0.9 to 1.5)	0.02	0.77
LDL cholesterol, mmol/L	2.4 (±0.7)	−0.12	0.15
Adjusted calcium, mmol/L	2.27 (±0.20)	−0.01	0.89
Phosphate, mmol/L	1.97 (±0.52)	−0.2	0.014
C-reactive protein, mg/L	1.0 (0.5 to 3)	0.04	0.61
Intact-PTH, pg/mL	146 (89 to 248)	−0.20	0.013
Ankle–brachial index	1.1 (1.0 to 1.2)	−0.22	0.0063
Pulse wave velocity, m/s	17.1 (14.5 to 20.2)	0.33	0.0001

Data are mean±SD or median (interquartile range) for continuous variables and percentage for categorical variables

Pulse wave velocity was assessed only in patients with normal ankle–brachial index defined as >0.9 (n=138)

to assess independent predictors of OPG, PWV, and clinical history of CVD. For these analyses, age, dialysis vintage, BMI, albumin, total cholesterol, phosphate, and intact-PTH were dichotomized according to median values. Restricted cubic splines were used to evaluate nonlinear relationships between OPG levels and outcome [23]. We chose four knots at quartiles, which have been suggested to offer adequate fit of the model and are good compromise between flexibility and loss of precision caused by overfitting a small sample. Thereafter, univariate and multivariate Cox regression analyses were presented as hazard ratio (HR) and 95% confidence interval (CI), using OPG concentrations as a continuous variable. All statistical analyses were performed using statistical software SAS version 9.2 (SAS Campus Drive, Cary, North Carolina, USA).

Results

Clinical characteristics and correlates of osteoprotegerin levels

Clinical characteristics of the 151 HD patients included in the study are summarized in Table 1. The levels of OPG

Table 2 Multivariate regression model predicting for osteoprotegerin (pmol/L) at time of inclusion in 151 hemodialysis patients

Parameter	Parameter Estimate	Standard Error	P value
Intercept	9.080	1.491	<0.0001
Age, >61 years	1.973	0.937	0.036
Sex, male	0.531	0.814	0.51
Dialysis vintage, >84 months	4.063	0.825	<0.0001
Diabetes mellitus, presence	0.208	0.870	0.81
Clinical history of CVD, presence	3.330	0.831	<0.0001
Body mass index, >20.9 kg/m^2	0.558	0.796	0.48
Albumin, >40 g/L	−1.072	0.839	0.20
Total cholesterol, >4.3 mmol/L	−0.088	0.820	0.91
Phosphate, >1.97 mmol/L	−1.009	0.838	0.23
Intact-PTH, >144 pg/ml	−2.028	0.772	0.0095

The adjusted r^2 of the model was 0.31

Categories for age, dialysis vintage, body mass index, albumin, total cholesterol, phosphate, and intact-PTH were calculated according to the median value of the group

presented a median value of 10.5 (25th–75th percentiles 7.3 to 15.1) pmol/L. Univariate correlations as assessed by Spearman's rank test are also shown in Table 1. Briefly, OPG levels were positively associated with age, dialysis vintage, clinical history of CVD and PWV, and negatively associated with body mass index (BMI), albumin, total cholesterol, phosphate, intact-PTH and ABI. CRP values were very low for all patients and did not associate with OPG concentration.

Using variables associated with OPG in univariate analysis, we performed a multivariate regression predicting for OPG (pmol/L; Table 2). Results showed independent associations of OPG with age, dialysis vintage, clinical history of CVD, and intact-PTH, but not with sex, diabetes mellitus, BMI, albumin, total cholesterol, and phosphate.

Patients with a clinical history of CVD had higher OPG levels as compared with those with no history of CVD (13.6 [8.6 to 18.5] vs. 9.4 [6.5 to 13.4] pmol/L; $P<0.001$). In a logistic regression model, such association remained and was independent of age, sex, dialysis vintage, and diabetes (Table 3).

One hundred and 38 patients had a normal ABI, defined as ABI>0.9 [21] and PWV was assessed in those. In these 138 patients, OPG levels were positively correlated to PWV (Table 1 and Fig. 1). The association between PWV (m/s) and OPG (pmol/L) was independent (estimate for OPG=0.169, Standard error=0.071, $P=0.018$) after inclusion of age, sex, dialysis vintage, and diabetes mellitus in a multivariate regression model (Table 4).

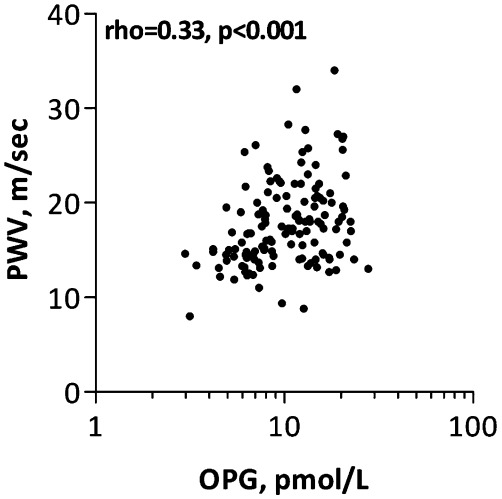

Fig. 1 Spearman Rank's correlation between plasma *OPG* concentrations and pulse wave velocity (*PWV*). One hundred and thirty-eight patients out of 151 patients had a normal ankle–brachial pressure index (ABI), defined as ABI>0.9 and therefore PWV could be assessed. Spearman's ρ (*rho*)=0.33, $P<0.001$

Osteoprotegerin levels and clinical outcome

During 6 years of follow-up, 40 patients died due to cardiovascular causes [$n=25$: acute myocardial infarction ($n=10$), chronic heart failure ($n=5$), dissecting aortic aneurysm ($n=1$), cerebral infarction ($n=6$), cerebral hemorrhage ($n=3$)], malignancies ($n=3$), infectious diseases ($n=7$) and other ($n=5$). Three patients who underwent kidney transplantation and two patients who were transferred to another hospital were censored. When presenting data as an age- and sex-adjusted restricted cubic spline curve, we could observe that increasing OPG concentrations had an impact on all-cause mortality (Fig. 2). Cox regression analysis revealed that patients with high OPG levels had increased all-cause and CVD-related mortality, which persisted after adjustment for age, sex, dialysis vintage, diabetes mellitus, and clinical history of CVD (Table 5).

Table 3 Odds ratios and 95% CI for factors predicting the presence of clinical history of CVD at the time of inclusion in 151 hemodialysis patients

Parameter	Odds ratio (95% Cl)	*P* value
Intercept		<0.0001
Age, >61 years	1.30 (0.57 to 2.94)	0.53
Sex, male	1.23 (0.58 to 2.61)	0.59
Dialysis vintage, >84 months	0.35 (0.14 to 0.87)	0.023
Diabetes mellitus, presence	1.59 (0.72 to 3.53)	0.25
OPG, pmol/L	1.15 (1.07 to 1.25)	0.0004

Output of a logistic regression model with absence of clinical history of CVD taken as the reference

Categories for age and dialysis vintage were calculated according to the median value of the group

The r^2 of the model was 0.15

Table 4 Multivariate regression model predicting for PWV (m/s) at time of inclusion in 138 hemodialysis patients

Parameter	Parameter Estimate	Standard Error	*P* value
Intercept	14.041	0.937	<0.0001
Age, >61 years	3.795	0.749	<0.0001
Sex, male	−0.655	0.688	0.34
Dialysis vintage, >84 months	0.311	0.781	0.69
Diabetes mellitus, presence	0.774	0.772	0.32
OPG, pmol/L	0.169	0.071	0.018

The adjusted r^2 of the model was 0.23

Pulse wave velocity was assessed only in patients with normal ankle–brachial index defined as >0.9 ($n=138$)

Categories for age, dialysis vintage, body mass index, and intact-PTH were calculated according to the median value of the group

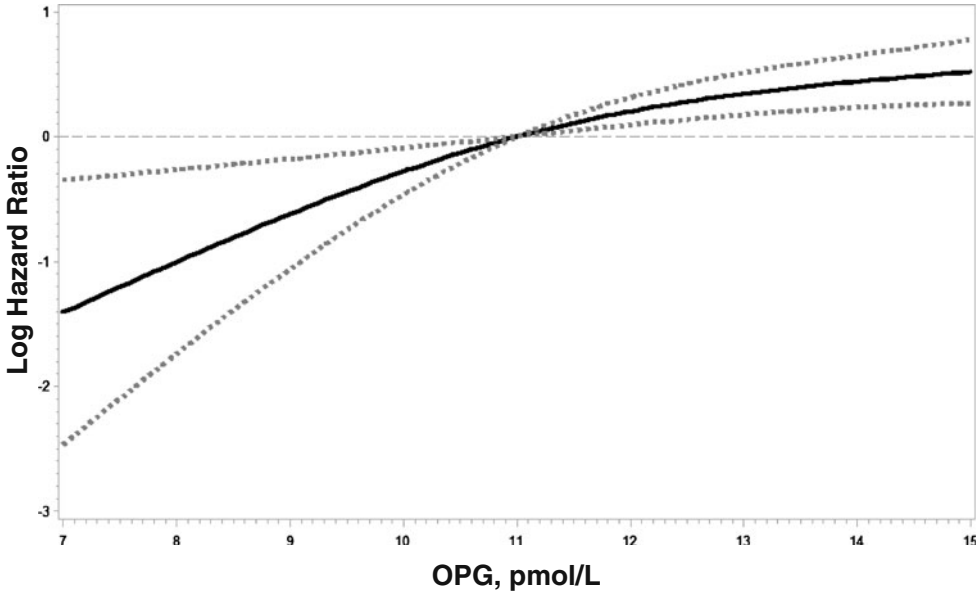

Fig. 2 Spline curve showing log-transformed hazard ratios and 95% confidence intervals (*dashed lines*) for all-cause mortality associated with OPG values in 151 hemodialysis patients. The model is plotted as restricted cubic splines with four knots and adjusted for age and sex. *P* for linearity=0.08

Discussion

This is the first report linking high OPG concentrations with increased mortality in Japanese HD patients. At the time of study initiation, there was no evidence linking OPG concentrations and mortality in dialysis patients. However, during the follow-up period several reports have now documented such relationship in both chronic HD [11, 12] and pre-dialysis CKD stages [15, 24, 25]. It is interesting to pinpoint, however, that while previous studies have suggested that OPG concentrations are influenced by residual renal function [8], malnutrition [17], inflammation [11, 15], and intravenous maxacalcitol therapy [18], none of these conditions were present in our patient population. Thus, a strength of our study is that our conclusions are based on less biased and therefore more robust associations between OPG, arterial stiffness, and outcome. Our results also further reinforce the involvement of a high OPG concentration in the mortality risk of HD patients by the demonstrated association with both the presence of clinically evident CVD and arterial stiffness.

It should be noted that these associations were statistically significant despite the low mortality risk of Japanese as compared to Western HD patients. The mortality rate of our patients (4.4/100 patient years) agrees with, but is even lower, than the reported Japanese mortality rate for HD patients (6.6/100 patient years) [14], presumably attributed to the exclusion of hypoalbuminemic patients. Another phenotypical feature which deviates from typical findings in Western HD patients is that CRP had a median value of 1.0 mg/L, in line with the lower prevalence of inflammation in Japanese CKD patients [16]. Thus, the patients in the current study are unlikely to present with the malnutrition, inflammation, and atherosclerosis syndrome. Recent studies suggested that OPG levels were associated to mortality only in the presence of concurrent inflammation [11, 15], leading to the hypothesis that low-grade persistent inflammation serves as a catalyst and modifies/exacerbates the atherosclerosis process [15, 26]. However, the current study cannot confirm this hypothesis as OPG was related to increased mortality risk in a group of well-nourished and uninflamed HD Japanese patients.

Table 5 Cox regression analysis for osteoprotegerin levels regarding all-cause and cardiovascular-related mortality in 151 hemodialysis patients

Model	Covariates	All-cause mortality		Cardiovascular mortality	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
1	Crude, 1 pmol/L	1.14 (1.08–1.20)	<0.0001	1.14 (1.07–1.21)	<0.0001
2	1+age and sex	1.14 (1.08–1.20)	<0.0001	1.14 (1.06–1.22)	0.0002
3	2+dialysis vintage, diabetes, and baseline CVD	1.12 (1.05–1.19)	0.0004	1.11 (1.02–1.19)	0.011

Indicated are crude HRs (model 1) or with various degrees of adjustment (models 2 and 3) for all-cause and CVD-related mortality according to OPG levels

Categories for age and dialysis vintage were calculated according to the median value of the group

Our study also shows that OPG in multivariate analyses was associated with arterial stiffness as well as both clinically evident CVD and CVD mortality. Extending this, a recent Japanese report associated OPG concentrations with the prediction of non-fatal CVD events in HD patients [27], while others have suggested similar associations using different surrogates of arterial calcification in chronic HD patients [9, 10, 12]. The cross-sectional nature of this analysis precludes us from inferring whether these factors are causally linked. However, the hypothesis that OPG may be causing vascular calcification in these patients by inhibiting bone remodeling is not consistent with the known actions of OPG. In animal models, absence of OPG increases vascular calcification [6], while excess levels reduce vascular calcification [7] even in the face of acute challenges [28]. In addition, the circulating levels of OPG observed are likely too low to have a systemic effect and may reflect low levels of escape from local tissue sources, perhaps vessels themselves. Thus, it would seem more likely that the observed association with vascular mortality represents a compensatory, rather than causative relationship.

Some limitations of the present study should be noted, including the abovementioned cross-sectional design, and the fact that fatal cardiovascular events are recorded based on patient records and not confirmed by autopsies, probably underestimating the true prevalence of cardiac endpoints. Another limitation is the lack of repeated measurements of both OPG levels and PWV during follow-up, which would have enabled us to make a stronger case. In summary, we report that in normoalbuminemic Japanese HD patients with no apparent inflammation, circulating OPG levels are independent prognosticators of all-cause and CVD-related mortality, being closely related also to the degree of arterial stiffness. These conclusions, while in agreement with observations in non-Japanese and thus ethnically different CKD populations, are based on more unbiased relationships and strengthened by the fact that several reported confounders of OPG levels, such as malnutrition or inflammation were excluded or not present.

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Conflicts of interest BL is employed by Baxter Healthcare, Inc. No other author declares a personal or financial conflict of interest.

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