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ORIGINAL ARTICLE

Relationship between serum osteoprotegerin and vascular calcifications in hemodialysis patients

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Abstract *Background:* Uremia is a vasculopathic process, and both cardiac calcification and vascular calcification seen from the early stages of chronic kidney disease. Osteoprotegerin could play a crucial role in atherosclerotic plaque formation, maturation and calcification. The goal of this study was to determine the relationship of serum osteoprotegerin with vascular calcification in patients with end stage kidney disease who were maintained on regular hemodialysis.

Methods: Sixty clinically stable chronic renal failure patients undergoing regular hemodialysis were enrolled in this cross sectional study. Thirty patients (mean age 56.7 ± 10.5 years) with abdominal aortic calcification were selected by basal abdominal X-ray who underwent multi-slice computerized tomography scan to measure coronary artery calcification score; and thirty patients (mean age 56.5 ± 8.4 years) without abdominal aortic calcification. All patients were evaluated by serum calcium, phosphorus, albumin, lipid profile, intact parathyroid hormone (iPTH), serum creatinine, serum urea, serum uric acid, serum C-reactive protein, and hemoglobin. Serum osteoprotegerin samples were collected before dialysis and estimated by the ELISA kit.

Results: Serum osteoprotegerin level was significantly higher in patients with vascular calcification than in those without calcifications. Serum osteoprotegerin correlated positively with serum phosphorus, calcium phosphorus product, alkaline phosphatase, iPTH, C-reactive protein, serum uric acid, low-density lipoprotein (LDL) and left ventricular mass index (LVMI) ($p < 0.005$), and negatively with hemoglobin, ejection fraction ($p < 0.005$) and HDL ($p < 0.05$).

Conclusions: These findings suggest that osteoprotegerin may be involved in the development of vascular calcification in hemodialysis patients.

1. Introduction

Vascular calcification (VC) is common in individuals with chronic kidney diseases.[1] Vascular calcification is highly

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prevalent in patients on dialysis as 94% of the patients had aortic calcification detected by lateral abdominal radiography.[2] Similarly, Jean et al.[3] reported a high prevalence 83% of vascular calcifications in hemodialysis (HD) patients in spite of a long and intensive dialysis strategy and adherence to guidelines. Emad et al. reported 78% of Egyptian HD patients showing abdominal aortic calcification.[4]

The pathogenesis of vascular calcifications is complex and not fully understood. It does not only consist of a simple precipitation of calcium and phosphorus, but also involves an active and modifiable process. Vascular calcification in chronic kidney diseases (CKD) may be interpreted as the result of the dysregulation of the equilibrium between calcification promoters and inhibitors in which several uremic factors, including abnormalities in the mineral metabolism, are implicated.[5]

Osteoprotegerin (OPG) is a cytokine of the tumor necrosis factor (TNF) receptor superfamily and is classed as an osteoclastogenesis inhibitory factor. OPG is expressed widely in many tissues besides osteoblasts, including spleen, bone marrow, heart, liver, and kidney.[6] The plasma level of OPG could serve as a surrogate marker of progression of atherosclerosis and calcification in patients with end-stage renal disease.[7]

It has also been demonstrated that serum Osteoprotegerin (OPG) levels are correlated with the severity of coronary artery disease and constitute an independent risk factor of the progression of atherosclerosis.[8] A study by Morena et al. suggests that there is a significant correlation between elevated serum OPG levels and cardiovascular mortality.[9]

We aimed to investigate the relation between osteoprotegerin level and cardiovascular calcification in hemodialysis patients.

2. Patients and methods

The study was carried out for a period of one year from January 2014 to January 2015 at El-Galaa Military Hospital. A total of 60 patients with clinically stable end stage kidney disease (ESKD) were enrolled in this cross-sectional study. According to lateral abdominal X-ray, we selected 30 patients with abdominal aortic calcification (group A) and they had been compared with another 30 patients selected without abdominal aortic calcification (group B). All patients were dialyzed via AVF three times a week for four hour session using polysulfone high flux dialyzer 1.6 m^2 surface area, with dialysate flow 500 ml/min and dialysate calcium concentration 1.25 mmol/l, using heparin as anticoagulant with tailored doses according to each case and bicarbonate based dialysate. The adequacy of dialysis was assessed using Kt/V formula (K is patient clearance, t dialysis time, V urea space).[10] Patients with the following criteria were included in the study: patients older than 25 years; parathyroid hormone (PTH) > 300 pg/ml; serum calcium > 8.4 mg/dl and calcium phosphorus product > 50 mg/dl. On the other hand, patients subjected to parathyroidectomy; severely anemic patients (Hb < 7 g/dl); patients with advanced cardiac dysfunction (EF% < 35%); patients on oral anticoagulants; and patients on regular hemodialysis less than 6 months and diabetics were excluded.

Each patient underwent full clinical evaluation, serum calcium, phosphorus, alkaline phosphatase albumin, lipid profile, PTH, serum creatinine, serum urea, Serum uric acid, C-reactive protein (CRP), and hemoglobin. Osteoprotegerin samples were collected before dialysis and stored at −80 °C until use. Serum OPG samples were estimated by the ELISA kit provided by Phoenix Pharmaceuticals, Prague-Czech. All patients were informed about the content of the study and gave their written approvals before enrollment. All procedures were performed in accordance with the ethical standards of Al-Azhar University's committee on human experiments.

Radiological evaluation: It was performed through Echocardiography to measure different cardiac parameters especially left ventricular diameters and ejection fraction. Lateral abdominal X-radiography of the aorta for grading of calcifications was as follows: 0, no aortic calcific deposits; 1, small scattered calcific deposits less than one-third of the corresponding length of the vertebral level; 2, medium quantity of calcific deposits about one-third or more, but less than two-thirds of the corresponding vertebral length; 3, severe quantity of calcifications of more than two-thirds or more of the corresponding vertebral lengths. To detect abdominal aortic calcification we used a validated 24-point abdominal aortic calcification score (AACS). For the 24-point score, calcified deposits along the anterior and posterior longitudinal walls of the abdominal aorta adjacent to each lumbar vertebra from L1 to L4 were assessed using the midpoint of the intervertebral space above and below the vertebrae as the boundaries. The scores, obtained separately for the anterior and posterior walls, resulted in a range from 0 to 6 for each vertebral level and 0 to 24 for the total score.[11] Multi-slice CT scan (MDCT) was used to measure coronary artery calcification score using Agatston score. The score was calculated using a weighted value assigned to the highest density of calcification in a given coronary artery. The density was measured in Hounsfield units, and score of 1 for 130–199 HU, 2 for 200–299 HU, 3 for 300–399 HU, and 4 for 400 HU and greater. This weighted score was then multiplied by the area (in square millimeters) of the coronary calcification. The tomographic slices of the heart were 3 mm thick and average about 64 slices from the coronary artery ostia to the inferior wall of the heart. The calcium score of every calcification in each coronary artery for all of the tomographic slices was then summed up to give the total coronary artery calcium score (CAC score).[12]

3. Statistical methods

All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows. Data are presented as mean and SD. In addition, parametric tests for comparison of numerical variables between the study groups were done using Independent Samples T Test. For comparing categorical data Chi-squared test was performed. Correlation between numeric variables was done using Pearson's correlation equation. Stepwise multiple regression analysis was for detection of vascular calcifications predictors. P values less than 0.05 was considered statistically significant.

4. Results

Characteristics of study groups are summarized in Table 1. Both groups were matched regarding age, gender, body mass index (BMI), original kidney disease. The patients with vascular calcifications have higher duration of hemodialysis, serum

Table 1 Demographic, laboratory and echocardiographic data of the patients.

	Group A (n = 30)	Group B (n = 30)	P value
Mean age (years) ± SD	56.7 ± 10.5	56.5 ± 8.4	>0.05
Sex (male/female)	13/17	14/16	>0.05
Duration of hemodialysis/month (mean ± SD)	76.9 ± 29.2	34.5 ± 16.7	<0.005
BMI g/m^2 (mean ± SD)	26.6 ± 4.3	28 ± 3.4	>0.05
Original kidney disease (n &%)			
Hypertension	11(36.7%)	9(30%)	
Chronic pyelonephritis	5(16.7%)	4(13.3%)	
Chronic glomerulonephritis	5(16.7%)	7(23.3%)	
Obstructive uropathy	4(13.3%)	2(6.7%)	>0.05
APCKD	2(6.7%)	3(10%)	
Unknown	3(10%)	5(16.7%)	
OPG (pg/ml) (M ± SD)	714.30 ± 433	332.36 ± 176	<0.001
OPG (pg/ml)			
Male	477 ± 373		
Female	560 ± 388		>0.05
Creatinine (mg/dl) (M ± SD)	8.6 ± 1.7	8.8 ± 1.5	>0.05
Urea (mg/dl) (M ± SD)	174 ± 30	167 ± 40	>0.05
Kt/V(M ± SD)	1.24 ± 0.1	1.28 ± 0.12	>0.05
Hemoglobin (g/dl) (M ± SD)	9.45 ± 0.72	10.02 ± .61	>0.05
C-reactive protein (M ± SD)	5.26 ± 1.8	3.46 ± 0.92	<0.001
Serum phosphorus (mg/dl) (M ± SD)	6.2 ± 0.77	5.7 ± .0.53	<0.001
Serum calcium (mg/dl) (M ± SD)	9.6 ± 0.8	9.4 ± 0.7	>0.05
Serum albumin(g/dl) (M ± SD)	3.56 ± 0.55	3.61 ± 0.53	>0.05
Ca-Ph product (mg/dl) (M ± SD)	59.6 ± 4.8	54 ± 3.9	<0.001
Serum PTH(pg/ml) (M ± SD)	550 ± 180	471 ± 160	>0.05
Alkaline phosphatase (IU/dl) (M ± SD)	163.6 ± 30	155.7 ± 20	>0.05
Serum cholesterol (mg/dl)	225 ± 21	206 ± 50	>0.05
Serum triglycerides (mg/dl)	162 ± 25	164.9 ± 25	>0.05
Serum LDL (mg/dl) (M ± SD)	136 ± 21	129 ± 22	>0.05
Serum HDL mg/dl) (M ± SD)	40 ± 6.5	39.9 ± 5.5	>0.05
Serum uric acid (mg/dl) (M ± SD)	6.4 ± 1.3	5.6 ± 1.1	<0.05
Left ventricular mass index (g/m^2) (M ± SD)	265.4 ± 41.3	214.6 ± 50	<0.001
Ejection fraction % (M ± SD)	56.1 ± 6	62.4 ± 6.9	<0.001

phosphorus, calcium-phosphorus product, CRP and serum uric acid. Also, the patients with vascular calcifications have high left ventricular mass index (LVMI) and low ejection fraction (IF). These changes are associated with increased OPG levels in patients with vascular calcifications (Table 1). There are positive correlations between OPG and age, duration of hemodialysis, serum phosphorus, calcium phosphorus product, alkaline phosphatase, PTH, CRP, serum uric acid, and LDL and there is negativity with serum calcium. In addition, OPG correlated positively with AACS, CACS and LVMI while negatively with ejection fraction (Table 2). Stepwise multiple regression analysis by using of AACs and CACs as dependent variables separately revealed that the predictors of both abdominal aortic calcifications and coronary artery calcifications are CRP, cholesterol and alkaline phosphatase levels (Tables 3, 4), and the remaining variables are excluded.

5. Discussion

Several mechanisms have been implicated for the high prevalence of vascular calcification in CKD, including the high burden of conventional cardiovascular risks such as hypertension, dyslipidemia, bone and mineral disorders such as calcium load and secondary hyperparathyroidism (SHPT), Chronic inflammation such as high level of pro inflammatory cytokines and deficiency of anticalcemic factors.[8]

In this current study, we found that all patients with abdominal aortic calcification have coronary artery calcification and its extent directly correlated with abdominal aortic calcification. The patients with vascular calcification have higher serum phosphorus and calcium phosphorus (Ca × P) product than those without vascular calcifications. These results agree with several studies. Elevated serum phosphorus is now recognized as a major risk factor for cardiovascular events in stage 4 chronic kidney disease (CKD 4) and the general population.[13] Mortality in ESKD patients is strongly correlated with serum phosphorus levels >5.5 mg/dl.[14] Also, relatively small elevations in serum phosphorus in the high normal range (3.5–4.5 mg/dl) have been correlated with increased risk of cardiovascular and all-cause mortality in CKD patients and the general population with normal renal function.[15] In addition, several studies that concluded that, an increased calcium phosphorus product and hyperphosphatemia are thought to be a key determinant of cardiovascular mortality and progression factors of unwanted calcifications in uremia.[16–20]

The role of PTH is also unclear, PTH Fragments 1–34 have been shown to inhibit calcification in a murine model of atherosclerotic vascular calcification[21] but PTH 7–84 may act

Table 2 Correlations between OPG, AACS and CACS other laboratory variables.

	Aortic calcification score		Coronary calcification score		OPG (pg/dl)	
	R	Sig.	R	Sig.	R	Sig.
Age	.585**	.001	.516**	.004	.371**	.004
Duration of HD	.728**	.000	.734**	.000	.727**	.000
BMI	.290	.120	.301	.106	.086	.516
Serum creatinine	−.203	.282	−.173	.361	−.103	.432
Blood urea	−.166	.379	−.231	.218	−.087	.507
Kt/V	−.167	.378	−.190	.315	−.086	.513
Serum Phosphorus	.563**	.001	.420*	.021	.651**	.000
Serum calcium	−.584**	.001	−.508**	.004	−.342**	.008
ca × po4 product	.240	.201	.105	.581	.494**	.000
Serum alkaline phosphatase	.743**	.000	.715**	.000	.505**	.000
PTH	.612**	.000	.461*	.010	.541**	.000
Serum albumin	−.086	.652	−.082	.667	−.177	.177
Serum cholesterol	.407*	.026	.384*	.036	.239	.066
Serum triglycerides	.364*	.048	.432*	.017	.007	.959
LDL	.597**	.000	.519**	.000	.359**	.005
HDL	−.468**	.009	−.381*	.038	−.278*	.031
Hb %	−.234	.069	−.196	.232	−.269	.058
CRP	.819**	.000	.760**	.000	.603**	.000
Serum uric acid	.285	.127	.298	.110	.343**	.007
Ejection fraction	−.624**	.000	−.507**	.004	−.494**	.000
LVMI	.624**	.000	.436*	.016	.609**	.000
Aortic calcification score					.788**	.000
Coronary calcification score					.626**	.000

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 3 Stepwise multiple regression analysis by using all tested variables and abdominal aortic calcification score as dependent variables.

Model		Unstandardized coefficients		Standardized coefficients	*t*	Sig.
		B	Std. error	Beta		
1	(Constant)	−1.299	1.202		−1.080	.289
	CRP	1.634	.216	.819	7.552	.000
2	(Constant)	−14.348	3.524		−4.071	.000
	CRP	1.578	.177	.791	8.893	.000
	Serum cholesterol (mg/dl)	.059	.015	.343	3.856	.001
3	(Constant)	−18.498	3.479		−5.317	.000
	CRP	1.092	.235	.547	4.644	.000
	Serum cholesterol (mg/dl)	.061	.014	.353	4.442	.000
	Serum alkaline phosphatase (IU/dl)	.039	.014	.329	2.801	.009

to increase vascular calcification[22] and high PTH levels are often associated with high calcification scores.[23] In the current study, serum calcium, PTH, and alkaline phosphatase of patients with VC and those without VC were a like comparable and this in concordance with Graciolli et al.[24] demonstrated that PTH itself is not able to induce vascular calcification but has a synergistic effect with the phosphorus probably due to an indirect and deleterious effect associated with bone remodeling and osteoclastic activity. In addition, Kazuhiro et al.[25] found that serum calcium, serum phosphorus and the calcium phosphorus product were not associated with aortic calcification and this may be related to the overall good mineral control of their patients.

In addition, we found significantly high CRP and serum uric acid among patients with vascular calcifications. It is well known that inflammation has a key role in coronary artery disease and other manifestations of atherosclerosis.[26] CRP was significantly associated with the presence of vascular calcification in both aorta and hand arteries.[27] Serum uric acid above 6 mg/dl is associated with an increased risk of calcification and cardiovascular adverse events in CKD patients in dialysis,[28] while Caliskan et al.[29] and Turkmen et al.[30] found no correlation between hs-CRP levels and CACS.

Several epidemiological studies suggested that elevated serum levels of OPG were associated with the vascular risk and calcification.[31,32,9,33–35] In the current study the levels of

Table 4 Stepwise multiple regression analysis by using all tested variables and coronary artery calcification score as dependent variable.

Model		Unstandardized coefficients		Standardized coefficients	*t*	Sig.
		B	Std. error	Beta		
1	(Constant)	−334.350	242.299		−1.380	.179
	CRP	269.794	43.597	.760	6.188	.000
2	(Constant)	−2532.703	766.913		−3.302	.003
	CRP	260.487	38.623	.734	6.744	.000
	Serum Cholesterol (mg/dl)	9.966	3.339	.325	2.985	.006
3	(Constant)	−3343.456	778.820		−4.293	.000
	CRP	165.426	52.642	.466	3.142	.004
	Serum Cholesterol (mg/dl)	10.313	3.071	.336	3.358	.002
	Serum alkaline phosphatase (IU/dl)	7.534	3.081	.362	2.445	.022

circulating OPG are significantly higher among hemodialysis patients with vascular calcifications. The mechanism that can explain the association of elevated OPG levels with vascular calcification in hemodialysis is still controversial. It can be elevated as a result of vascular damage,[31] or it is elevated as it has a role in calcification process itself,[33] and it may be as a compensatory protective response to progression of vascular calcification. Also, it was stated that circulating OPG level in CKD patients may indicate a state of resistance to OPG action due to the uremic condition,[22] or it may be uremic toxin that increases the skeletal resistance to PTH.[32,9] The elevated OPG dramatically reduced after successful renal transplantation.[36]

Moreover, we noticed that OPG levels are related to age, duration of hemodialysis, calcium, phosphorus, alkaline phosphatase, calcium phosphorus product, PTH, CRP, HDL and LDL. Similarly, Ozkok et al.[37] reported that serum OPG levels were significantly correlated with age and duration of dialysis and serum albumin levels. Azar et al. reported the relationship of OPG with age, calcium levels while PTH was not shown to be significant.[38] The association of CRP with OPG has been reported by Kiech et al.[39] in which serum CRP levels had a positive correlation with serum OPG levels. Kendrick and Chonchol[40] found a positive weak correlation between OPG and both total cholesterol and triglycerides only in the obese, and these correlations disappeared after adjustment for age. Junichiro et al.[32] and Morena et al.[33] found no significant correlation between OPG level and calcium and phosphate, PTH but the low number of patients lessens the strength of this study.

In the present study, the predictors for the presence of abdominal aortic calcifications and coronary artery calcifications are CRP, cholesterol and alkaline phosphatase. There are several lines of evidence showing that CAC is associated with systemic inflammation[41–44] as the component of the mal nutrition-inflammation-atherosclerosis syndrome. Atherosclerosis frequently coexists with systemic inflammation especially in hemodialysis diabetic patients.[45]

Abdominal aortic calcification deposits are a marker of subclinical atherosclerotic disease and an independent predictor of subsequent vascular morbidity and mortality and the predictors for the presence and severity of abdominal aortic calcification were age, duration of dialysis and history of cardiovascular disease.[46] The results of Pencak et al.'s study demonstrated that AAC preceded the occurrence of CAC and revealed differences in factors favoring their development. Age and hemodialysis vintage were equally (OR = 1.13 and 1.14) important for the occurrence of CAC, while only age was a major (OR = 1.20) predictor of the occurrence of AAC.[47]

AACS have positive correlations with age, duration of hemodialysis, serum phosphorus, corrected serum calcium and calcium-phosphorus product, iPTH, cholesterol, triglycerides, low density lipoprotein (LDL), CRP and serum uric acid, and negative correlation with high density lipoprotein (HDL). Moldovan et al.[48] found that the presence of vascular calcification was associated with age, gender, diabetes, dialysate calcium, serum calcium and vitamin D treatments, calcium - phosphorus product, PTH and CRP. Also, Nigel et al.[2] reported that the presence and severity of AAC were related to age, duration of dialysis and the presence of cardiovascular disease, but there was no significant relationship between AACS and serum markers of mineral metabolism (phosphate, calcium, PTH, 25-hydroxy vitamin D), lipids, C-reactive protein or the presence of diabetes. Moreover, Nishizawa et al.[49] concluded that the significant factors affecting vascular calcification were advanced age, longer duration of hemodialysis, increased phosphate concentrations and lower predialysis diastolic pressure.

In the current study, CACS directly correlated with age, duration of hemodialysis, phosphorus, calcium, PTH, alkaline phosphatase and CRP, cholesterol, triglycerides, LDL, and inversely with HDL. These results have been documented by many studies[16,33–35,22]. In contrast, Nitta et al.[42], Berenzin and Kremzer[50] reported that the CACS had no significant correlation with serum calcium, serum phosphorus and PTH. The heterogeneity of the study population, study design as single baseline laboratory value may lessen the strength of some of the significant predictors of CAC especially those that vary widely day-to-day.

Aortic calcification scores and coronary artery calcification scores correlated positively with OPG levels among our patients. Our results supported by many studies noticed strong association between OPG levels and vascular calcification in coronary arteries in patients with CKD[31,33,51] as well as in the aorta for patients with ESRD[3,48]. Both progression of vascular calcification[52,53] and cardiovascular events are related to high level of OPG in patients with CKD.[33,54,55]

6. Conclusions

Our results suggested that OPG may be involved in progression of vascular calcification in patients undergoing hemodialysis. Since, we live in the era of continuously emerging biomarkers for detection of cardiovascular calcification that differs in their sensitivity and specificity. Therefore, serum OPG can be used as recent, simple, easily performed non-invasive biomarker mirroring vascular calcification in hemodialysis patients as it is parallel with disease progression and it can be added to a panel or scoring system that might present a practical and non-invasive tool to detect cardiovascular calcification in hemodialysis patients.

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Conflict of interest

The authors declare that there is no conflict of interest.

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