



Vascular calcification is not related to serum fetuin-A and osteopontin levels in hemodialysis patients

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Abstract

Introduction Vascular calcification (VC) in hemodialysis (HD) patients is a sign of severe cardiovascular disease and can predict cardiovascular outcomes. Fetuin-A and osteopontin (OPN) inhibit VC. Serum fetuin-A levels are lower in patients with end-stage kidney disease (ESKD) and in those who are on chronic HD therapy. However, there are limited data concerning OPN in patients who are on dialysis. The aim of our study was to determine VC in HD patients, the relationship between VC and 25-OH-vitamin D, fetuin-A, and OPN levels, and independent predictors of VC.

Materials and methods Ninety-three patients with ESKD on HD therapy were recruited. Among these patients, 44 were male and 49 were female. The patient group was compared with a group of 20 healthy controls of similar age and sex. A plain radiograph of the hand was taken using a mammography machine for the evaluation of VC. Serum fetuin-A, OPN, and 25-OH-vitamin D levels of both patients and controls were measured.

Results VC was detected in 45 (48.4%) HD patients. When patients were compared with healthy controls, fetuin-A levels ($p < 0.029$) were significantly lower in patients, whereas OPN ($p < 0.000$) and VC ($p < 0.002$) were significantly higher in the patient group. Age [odds ratio (OR) 1.036], the presence of diabetes mellitus (DM) (OR 17.527), and high parathyroid hormone (PTH) levels (OR 1.002) were independent predictors of VC in a logistic regression model including the following factors: age, the presence of DM, HD duration, and serum albumin, phosphate, PTH, 25-OH-vitamin D, fetuin-A, OPN, and calcium levels. No significant correlation was found between patients with VC and patients without VC in terms of fetuin-A, OPN, and 25-OH-vitamin D levels.

Conclusions VC is a frequent sign in patients undergoing HD and is not related to serum fetuin-A and osteopontin levels. Age, the presence of DM, and high PTH levels were independent predictors of VC in patients undergoing HD. Further studies are warranted to understand the mechanism underlying and the factors contributing to VC.

Keywords Vascular calcinosis · Fetuin-A · Osteopontin · Hemodialysis

Introduction

Cardiovascular disease remains the leading cause of mortality and morbidity in end-stage kidney disease (ESKD) patients. Indeed, it is responsible for 20% of hospitalizations and 50% of mortality in ESKD patients and the cardiovascular disease (CVD)-related mortality rate is 10–20 times higher in these patients compared to the normal population [1, 2].

In addition to the traditional risk factors, such as older age, hypertension (HT), diabetes mellitus (DM), and dyslipidemia, which contribute to the pathogenesis of atherosclerosis in ESKD patients, several risk factors related to uremia exist as well, including inflammation, oxidative stress, mineral-bone disorders, hypervolemia, and uremic toxins.

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Recent studies suggest vascular calcification (VC) as a risk factor for CVD and phenotypic differentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells as the chief underlying event. VC is a dynamic process influenced by mineral-bone disorders, as well as an imbalance between promoters and inhibitors of extra-osseous bone formation [3, 4].

Fetuin-A is a liver-derived plasma glycoprotein and a potent inhibitor of VC. It exerts its effects in the circulation and forms soluble complexes with calcium and phosphate [5]. Osteopontin (OPN) is a glycoprotein synthesized from several types of cell, including VSMCs and endothelial cells, which prevents calcification [6]. Numerous clinical studies have demonstrated that lower fetuin-A levels are associated with an increased VC and an increased risk of mortality, but little is known concerning OPN in the renal population.

Electron beam computed tomography (EBCT) and multislice computed tomography (CT) are frequently used, albeit expensive, methods for precise assessment of VC. The Kidney Disease Improving Global Outcomes (KDIGO) 2009 guidelines suggest the use of a more inexpensive method, namely lateral abdominal radiography, for the semiquantitative assessment of VC [7]; however, there is no consensus on the best choice for VC imaging. As a sensitive method to determine microcalcification, we used radiographs taken with a mammography machine to screen for VC.

The aim of this study was to determine the frequency of VC in hemodialysis (HD) patients using the mammography method, as well as to determine its relationship with fetuin-A, OPN, and 25-OH-vitamin D levels.

Materials and methods

Study population

Ninety-three patients on HD for more than 3 months, and 20 age-matched voluntary healthy controls, were included in this study. Patients were excluded if they had active infection, malignancy, chronic inflammatory disease (chronic pulmonary disease, chronic liver disease), or malnutrition, or were unable or unwilling to provide informed consent. No patient showed signs of inflammation or infection during the study period. Ethics approval was granted by the Inonu University Ethics Board (File No. 2012/154).

Data collection

Patient electronic records were used to obtain data on medical history, age, sex, cause of ESKD, comorbid conditions, and laboratory values before an HD session at the time of recruitment. Blood urea nitrogen (BUN), creatinine, albumin, calcium, phosphate, and parathyroid hormone (PTH)

levels of healthy controls were measured at the Inonu University biochemistry laboratory using routine procedures. Body mass index (BMI) was calculated by dividing dry weight in kilograms by the square of the height in meters. Corrected serum calcium was calculated as follows: corrected calcium (mg/dl) = serum calcium (mg/dl) + 0.8 (4 – serum albumin (g/dl)).

Samples obtained to measure calcification markers were collected from the controls and patients before an HD session, processed, and stored in – 80 °C freezers. Calcification markers were measured using the sandwich enzyme immunoassay method. Fetuin-A (BioVendor) was measured in serum, and OPN (eBioscience Platinum ELISA) was measured in plasma. High-performance liquid chromatography (LC-20 AT, Shimadzu) was used to measure 25-OH-vitamin D levels.

Radiologic imaging of VC

Anteroposterior wrist radiographs of patients and controls were taken with a mammography machine (Mammomat Inspiration, Siemens) as it is more powerful than direct radiography to determine calcifications. A horizontal line through the center of the metacarpal bones was drawn. The areas above and below this line were considered as two separate fields. A score of 2, 1, and 0 was assigned if VC was found in both fields, one field, or no fields, respectively.

Statistical analysis

Descriptive data are reported as mean and standard deviation (SD) or median and quartiles, as appropriate, for continuous variables, or as frequency for ordinal or nominal data. Differences between patients and controls were assessed using the Mann–Whitney *U* test or Student's *t* test as appropriate. Correlations were measured using Spearman's correlation. All reported probability values are two-tailed, and a *p* value < 0.05 was considered statistically significant. Categorical data are expressed as percentages and compared using Chi-square analysis or the Wilcoxon test. Logistic regression analyses were performed to determine the independent predictors of VC. Analyses were performed using SPSS software (ver. 17.0; SPSS Inc., Chicago, IL, USA).

Results

Of 93 patients, the etiological cause of chronic kidney disease was DM in 30 (32.3%) patients, HT in 26 (28%) patients, chronic glomerulonephritis in 16 (17.2%) patients, of idiopathic origin in 11 (11.8%) patients, and due to other reasons, including polycystic kidney disease, amyloidosis, renovascular diseases, and postrenal diseases, in 10 (10.8%)

patients. In the patient group, the median predialysis medical treatment duration was 6 (0–240) months and the median HD therapy duration was 53 (3–211) months.

Patient group and healthy controls were similar in terms of age, sex, BMI, and serum 25-OH-vitamin D levels. Fetuin-A levels were lower (673.6 ± 202.4 ng/ml, $p = 0.029$), and OPN levels were higher (5.4 ± 3.5 ng/ml, $p = 0.000$), in the patient group. VC was detected in 45 (48.4%) patients and was significantly higher in patients group ($p = 0.002$). VC in one field was detected in 22 (23.7%) patients, while it was detected in two fields in 23 (24.7%) patients. Serum BUN ($p < 0.000$), creatinine ($p < 0.000$), phosphate ($p < 0.000$), and PTH ($p < 0.000$) levels were higher in patients group as expected. Serum Ca ($p = 0.001$) and albumin ($p < 0.000$) levels were higher in healthy controls (Table 1).

Age, the presence of DM, HD duration, serum albumin, phosphorus, PTH, 25-OH-vitamin D, fetuin-A, OPN, and calcium levels have been included to logistic regression analysis. Age (OR 1.036), the presence of diabetes (OR 17.527), and elevated PTH levels (OR 1.002) were independent predictors of VC (Table 2).

When we compared patients with VC and patients without calcification, BMI (27.4 ± 4.8 ; $p = 0.031$), BUN (57.2 ± 14 mg/dl; $p = 0.028$), and creatinine levels (7.4 ± 2.4 mg/dl; 0.010) were significantly lower in patients without calcification. Similar fetuin-A and OPN levels were noted when the two patient groups were compared according to the presence of VC. Both groups were similar in terms of sex, age, serum albumin, Ca, P, PTH, CaxP, and 25-OH-vitamin levels (Table 3).

When we compared patient group according to their VC scores (VC = 0, VC = 1, VC = 2), no significant difference

Table 2 Independent predictors of vascular calcification in the patient

	OR	<i>p</i>
Age	1.036	0.053
The presence of DM	17.527	0.000
PTH	1.002	0.010

was found in terms of fetuin-A and OPN levels. Both groups were similar in terms of sex, age, BMI, BUN, serum albumin, Ca, P, PTH, CaxP, and 25-OH-vitamin levels. Serum creatinine levels in VC = 0 group (8.6 ± 2.7 mg/dl) were significantly higher [VC = 0 – VC = 1 ($p = 0.038$), VC = 0 – VC = 2 ($p = 0.037$)] when compared with the other groups (Table 4).

There were a significantly higher number of diabetic patients with VC (26 patients, 28%) when we compared with non-diabetic patients with VC ($p = 0.000$), and there was no significant difference between diabetic patients and non-diabetic patients in terms of sex, age, BMI, BUN, creatinine, serum albumin, Ca, P, PTH, CaxP, 25-OH-vitamin, fetuin-A, and OPN levels (Table 5).

Discussion

VC was prevalent among our HD patients (48.4%). Regression analysis including age, the presence of DM, HD duration, and serum albumin, phosphate, PTH, 25-OH-vitamin D, fetuin-A, OPN, and calcium levels, showed that age, the presence of DM, and serum PTH levels were independent predictors of VC. Although HD patients had significantly

Table 1 Baseline characteristics of study participants

	Patients (<i>n</i> : 93)	Control group (<i>n</i> : 20)	<i>p</i>
Sex (male %)	44 (47.3%)	12 (60%)	0.305
Age (years)	58.3 ± 15.7	52.4 ± 10	0.086
BMI	26.4 ± 5.4	27.3 ± 3	0.217
BUN (mg/dl)	60.7 ± 15.9	12.6 ± 1.9	0.000
Creatinine (mg/dl)	8 ± 2.6	0.7 ± 0.1	0.000
Albumin (mg/dl)	3.6 ± 0.5	4 ± 0.2	0.000
Ca (mg/dl)	8.7 ± 1	9.2 ± 0.4	0.001
P (mg/dl)	5 ± 1.3	3.2 ± 0.5	0.000
PTH (mg/dl)	550.6 ± 512.1	63.5 ± 33.7	0.000
25-OH-vitamin D (μgr/l)	12.7 ± 9.9	12.2 ± 6.5	0.477
Fetuin-A (ng/ml)	673.6 ± 202.4	803.8 ± 277.4	0.029
OPN (ng/ml)	5.4 ± 3.5	2.3 ± 0.8	0.000
With calcification <i>n</i> (%)	45 (48.4%)	2 (10%)	0.002

BMI body mass index, BUN blood urine nitrogen, Ca calcium, P phosphate, PTH parathormone, OPN osteopontin

Table 3 Comparison of the patients according to the presence of VC

	With VC <i>n</i> : 45 (48.4%)	Without VC <i>n</i> : 48 (51.6%)	<i>p</i>
Sex (male %)	23 (24.7)	21 (22.6)	0.480
Age (years)	61.8 ± 12.3	55.1 ± 17.8	0.065
BMI	27.4 ± 4.8	25.5 ± 5.8	0.031
BUN (mg/dl)	57.2 ± 14	64 ± 17.1	0.028
Creatinine (mg/dl)	7.4 ± 2.4	8.6 ± 2.7	0.010
Albumin (mg/dl)	3.5 ± 0.4	3.6 ± 0.5	0.088
Ca (mg/dl)	9.2 ± 0.9	9 ± 0.9	0.421
P (mg/dl)	4.9 ± 1.5	5.1 ± 1.1	0.207
PTH (mg/dl)	587.9 ± 466.2	515.7 ± 554.5	0.108
25-OH-vitamin D (μgr/l)	12.9 ± 9	12.5 ± 10.8	0.539
Fetuin-A (ng/ml)	651.5 ± 188.3	694.3 ± 214.7	0.377
OPN (ng/ml)	5.4 ± 3.6	5.5 ± 3.5	0.788
CaxP	45.3 ± 14.8	45.3 ± 9.5	0.365

BMI body mass index, BUN blood urine nitrogen, Ca calcium, P phosphate, PTH parathormone, OPN osteopontin

Table 4 Comparison of the patients according to VC scores

	VC = 0 (<i>n</i> = 48) No calcification	VC = 1 (<i>n</i> = 22) One area	VC = 2 (<i>n</i> = 23) Two areas	<i>p</i>
Sex (male %)	21 (22.6)	7 (7.5)	16 (17.2)	0.032
Age (years)	55.1 ± 17.8	61 ± 13.9	62.6 ± 10.7	0.177
BMI	25.5 ± 5.6	28 ± 5.1	26.9 ± 4.6	0.090
BUN (mg/dl)	63 ± 17.1	58.7 ± 15.6	55.7 ± 12.5	0.078
Creatinine (mg/dl)	8.6 ± 2.7	7.6 ± 2.9	7.3 ± 1.8	0.038*
Albumin (mg/dl)	3.6 ± 0.5	3.6 ± 0.3	3.4 ± 0.4	0.147
Ca (mg/dl)	9 ± 0.9	9.3 ± 1.2	9 ± 0.6	0.147
P (mg/dl)	5.1 ± 1.1	4.9 ± 1.2	5 ± 1.8	0.429
PTH (mg/dl)	515.7 ± 554.5	692.9 ± 530.9	482.9 ± 374.2	0.119
25-OH-vitamin D (μgr/l)	12.5 ± 10.8	12 ± 8.6	13.8 ± 9.3	0.659
Fetuin-A (ng/ml)	694.3 ± 214.7	680.9 ± 166.6	623.4 ± 206.6	0.375
OPN (ng/ml)	5.5 ± 3.5	5.4 ± 3.7	5.3 ± 3.6	0.963
CaxP	45.3 ± 9.5	45.4 ± 11.9	45.2 ± 17.4	0.511

BMI body mass index, *BUN* blood urine nitrogen, *Ca* calcium, *P* phosphate, *PTH* parathormone, *OPN* osteopontin

* VC = 0 – VC = 1 (*p* = 0.038), VC = 0 – VC = 2 (*p* = 0.037)

Table 5 Comparisons of the diabetic and non-diabetic patients

	Diabetic patients <i>n</i> : 30 (32.3%)	Non-diabetic patients <i>n</i> : 63 (67.7%)	<i>p</i>
Sex (male %)	18 (19.4)	26 (28)	0.093
Age (years)	62.5 ± 9.1	56.3 ± 17.7	0.89
BMI	27 ± 4.6	26.2 ± 5.7	0.524
BUN (mg/dl)	57.3 ± 13.9	62.3 ± 16.7	0.156
Creatinine (mg/dl)	7.4 ± 1.9	8. ± 2.8	0.138
Albumin (mg/dl)	3.4 ± 0.5	3.6 ± 0.5	0.103
Ca (mg/dl)	9 ± 0.6	9.1 ± 1	0.698
P (mg/dl)	4.9 ± 1.6	5 ± 1.2	0.338
PTH (mg/dl)	412.6 ± 279.9	615.2 ± 581.1	0.323
25-OH-vitamin D (μgr/l)	13.6 ± 8.7	12.2 ± 10.4	0.233
Fetuin-A (ng/ml)	666.7 ± 223	676.8 ± 193.6	0.739
OPN (ng/ml)	4.6483 ± 3.3	5.8 ± 3.6	0.124
CaxP	42.3 ± 14.7	45.7 ± 10.4	0.215
With calcification <i>n</i> (%)	26 (28%)	19 (20.4%)	0.000

BMI body mass index, *BUN* blood urine nitrogen, *Ca* calcium, *P* phosphate, *PTH* parathormone, *OPN* osteopontin

higher OPN levels and lower fetuin-A levels compared to healthy individuals, VC is not related to serum OPN and fetuin-A levels.

VC is observed frequently in ESKD patients and is associated with increased morbidity and mortality. The mechanism underlying VC is multifactorial and not fully understood. Two forms of VC, with different underlying mechanisms, exist in CKD patients: intimal and medial calcifications. Medial calcification is more frequent in DM and CKD patients.

Plain radiography is a simple, inexpensive, and easily accessible method for diagnosing VC. A railway appearance on the arterial wall such as angiography performed, is suggestive of medial calcification, while patchy calcification foci suggest intimal calcification and atherosclerosis [8]. In our study, 48.4% of patients had medial calcification.

Rather than passive accumulation of calcium–phosphate crystals in tissues, VC in ESKD patients is an active process involving differentiation of VSMCs toward osteoblasts and is induced by many activating and inhibiting factors. As a

negative acute-phase reactant, fetuin-A is a potent inhibitor of VC. Low fetuin-A levels in dialysis patients are associated with VC and mortality. In our study, serum fetuin-A levels in the patient group were lower than those of healthy controls ($p < 0.029$). However, no significant correlation between serum fetuin-A levels and the severity of VC was found in the patient group. Fetuin-A levels are generally low in HD patients due to chronic inflammation. Many studies have shown an association between low serum fetuin-A levels and an increased VC [9–12], but others have not demonstrated such an association [13, 14].

OPN is an important molecule that prevents calcification. It is responsible for decreasing intracellular calcium levels and stimulating osteoclasts that attach to α -v β 3, which is a member of the integrin family and is found on the surface of osteoclasts. OPN leads to bone destruction, allowing an increased synthesis of carbonic anhydrase, which in turn creates an acidic microenvironment for bone resorption. Moreover, it can directly prevent mineralization attaching to apatite crystals. Macrophages, endothelial cells, and smooth muscle cells located around an atherosclerotic plaque produce OPN in proportion to the severity of the lesion. In previous experiments, severe aortic calcification was detected in matrix Gla protein (MGP) and OPN-deficient mice when compared with only MGP-deficient mice, and ectopic calcification regressed when OPN was injected in the MGP-OPN-deficient group [15–18].

A study conducted by Chin-Te Lee et al., which investigated 84 patients on HD, compared plasma OPN levels and VC in HD patients. In that study, patients were grouped according to the presence or absence of VC and were not compared with a control group. No significant correlation between OPN level and VC was shown [19]. Ramirez-Sendoval JC and coworkers compared the OPN levels between low VC score and high VC score groups in peritoneal dialysis patients, and they found no difference between groups [20]. In another study in chronic kidney disease stage 2–3 patients, OPN levels and aortic calcification scores were compared. No significant correlation was found [21]. Moreover, in our study, no correlation was found between OPN level and VC, but the OPN levels were significantly higher in the patient group than in the control group ($p < 0.000$). OPN is considered a calcification inhibitor; however, the results of our study are contradictory and warrant further exploration as to whether higher OPN levels are protective against VC, or represent reaction against VC development. Animal experiments have shown that OPN levels were locally increased in VC fields. In a study by Westenfeld et al., rats with nephrectomies fed with a high-phosphate diet had increased OPN levels after the development of VC to neutralize calcification, and OPN was defined as a marker of reactive macrophage activity [22]. In a study by Rivet et al., biopsies of eight

ESKD patients with calciphylaxis were stained with OPN dye; all biopsy tissues were positive for OPN. This result demonstrates that OPN levels increase locally in fields of calcification [23].

Rosenberg et al. [24] showed that OPN plasma levels were elevated in 420 chronic heart failure patients with left ventricular dysfunction and were also correlated with disease severity and risk of mortality. Moreover, OPN was a more powerful prognostic marker than *N*-terminal pro-hormone brain natriuretic peptide (NT-proBNP). Volume overload and systolic heart failure are common in HD patients. However, we did not evaluate the volume load of the patients in our study or perform echocardiography (ECHO). In our study, blood samples were taken immediately before HD sessions and it is possible that patients were more volume-overloaded at this period, which may explain our high OPN levels.

OPN is a peptide excreted by the kidneys; therefore, it may be present in higher amounts in HD patients. OPN is also a proinflammatory mediator involved in the immune system that is released from macrophages, neutrophils, and lymphocytes. It plays a role in neutrophil chemotaxis, cell adhesion, cytokine release, and apoptosis [25]. CKD alone is a source of inflammation. HD filters, dialysate exposure, and many other factors may also contribute to an increased inflammation in patients undergoing HD, which may subsequently increase OPN levels.

The role of OPN is well understood in bone formation and resorption, immune function, autoimmune diseases, solid tumors, and inflammatory bowel disease, but little is known concerning its role in VC in uremic patients. Thus, more clinical research is needed to evaluate the role of OPN in this setting.

The 25-OH-vitamin D levels were low in both the patient and control groups in this study. We performed our study during the winter months, and vitamin D deficiency is a public health problem in Turkey; these reasons likely underlie the low 25-OH-vitamin D levels in both groups.

Although our study had the limitations associated with a cross-sectional design, it also had strengths. To our knowledge, this is the first study that has compared the OPN levels of HD patients and healthy controls. The use of mammography as a direct radiography method increased the sensitivity of our VC diagnoses, and this was the first study to diagnose VC using mammography screening. However, the sensitivity of mammography for VC diagnosis should be compared with that of other advanced imaging modalities, such as EBCT and multislice CT.

In summary, our study showed that VC is common in HD patients, and that age, the presence of DM, and PTH levels are independent determinants of VC. Moreover, we showed that VC is not related to serum OPN and fetuin-A levels. Further studies are needed to better understand VC

in HD patients and to identify the underlying mechanism of action of VC.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This study was conducted on human participants. The study was approved by the Ethical Committee of Inonu University, Malatya, Turkey, and is supported by scientific research and the project's unit of Inonu University. File No. 2012/148.

Informed consent Informed consent was obtained from all participants included in the study.

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