ORIGINAL ARTICLE

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Coronary Calcification Is Associated With Lower Bone Formation Rate in CKD Patients Not Yet in Dialysis Treatment

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ABSTRACT

Vascular calcification is a strong prognostic marker of mortality in hemodialysis patients and has been associated with bone metabolism disorders in this population. In earlier stages of chronic kidney disease (CKD), vascular calcification also has been documented. This study evaluated the association between coronary artery calcification (CAC) and bone histomorphometric parameters in CKD predialysis patients assessed by multislice coronary tomography and by undecalcified bone biopsy. CAC was detected in 33 (66%) patients, and their median calcium score was 89.7 (0.4–2299.3 AU). The most frequent bone histologic alterations observed included low trabecular bone volume, increased eroded and osteoclast surfaces, and low bone-formation rate (BFR/BS). Multiple logistic regression analysis, adjusted for age, sex, and diabetes, showed that BFR/BS was independently associated with the presence of coronary calcification [$p = .009$; odd ratio (OR) = 0.15; 95% confidence interval (CI) 0.036–0.619]. This study showed a high prevalence of CAC in asymptomatic predialysis CKD patients. Also, there was an independent association of low bone formation and CAC in this population. In conclusion, our results provide evidence that low bone-formation rate constitutes another nontraditional risk factor for cardiovascular disease in CKD patients. © 2010 American Society for Bone and Mineral Research.

KEY WORDS: CHRONIC KIDNEY DISEASE; PREDIALYSIS; CORONARY CALCIFICATION; BONE MINERAL METABOLISM; BONE-FORMATION RATE

Introduction

Vascular calcification is a frequent finding in chronic kidney disease (CKD) patients on dialysis.[1,2] Recent studies have demonstrated that vascular calcification is a strong and independent prognostic marker of mortality in that population.[3,4]

The uremic environment seems to be favorable to the development and progression of vascular calcification, which is a regulated and active process similar to ossification.[5,6] Current data have shown that high serum phosphorus, calcium, and calcium-phosphorus product, as well as other selected components present in the uremic plasma, are involved in the pathogenesis of calcification.[7,8] In addition, renal osteodystrophy, a common complication in CKD patients, has been shown to be associated with vascular calcification in hemodialysis patients.[9,10]

Recently, vascular calcification has been identified in predialysis CKD patients.[11–13] The relationship between vascular calcification and bone disorders has not been investigated previously in this population. Therefore, the purpose of this study was to evaluate the relationship between coronary calcification and bone histomorphometric parameters in CKD patients not yet on dialysis.

Methods

Subjects and study design

This is a cross-sectional study of 50 asymptomatic predialysis CKD patients from an outpatient nephrology clinic in São Paulo, Brazil. All patients were older than 18 years of age; had 24-hour creatinine clearances of between 15 and 90 mL/m^2 per minute; had been followed by a nephrologist for at least 3 months; had not been receiving any phosphate binders, vitamin D analogues, or corticosteroids; and had no evidence of inflammatory, neoplastic, or infectious disease.

All selected patients underwent clinical and physical evaluation. Weight and height were used to calculate the body mass index (BMI). Previous cardiovascular disease was characterized by the presence of myocardial infarction, angina pectoris, coronary artery revascularization, ischemic stroke, or a positive

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diagnostic procedure result (e.g., stress test, coronary angiography, or radionuclide image). Laboratory tests, multislice coronary tomography (MSCT), and bone biopsy were performed within a period of 30 days after patient selection.

All patients signed an informed consent form. The study protocol was reviewed and approved by the local institutional ethics board.

Laboratory tests

Blood samples were drawn in a fasting state for the following laboratory tests: creatinine, ionized calcium, phosphorus, alkaline phosphatase (references ranges: <270 U/L for males, <240 U/L for females), intact parathyroid hormone (iPTH; Immulite Assay, DPC, Los Angeles, CA, USA; reference range 10 to 65 pg/mL. iPTH levels in pg/mL and ng/L are equivalent), 1,25-dihydroxyvitamin D [1,25$(OH)_2$D; radioimmunoassay, Gamacounter, Perkin Elmer, Brazil; reference range 15.9 to 55.6 pg/mL), and 25-hydroxyvitamin D [25(OH)D; radioimmunoassay, DiaSorin, Stillwater, MN, USA; reference range 18 to 62 ng/dL]. Twenty-four-hour urine was collected to determine creatinine clearance, which was corrected for body surface area. Hyperparathyroidism, hyperphosphatemia, and hypercalcemia were defined by Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines regarding bone metabolism in the different stages of CKD.[14] Vitamin D deficiency and insufficiency were diagnosed when 25(OH)D values were lower than 15 ng/mL and between 16 and 30 ng/mL, respectively.[14]

Coronary tomography

The coronary artery calcification score (CaSc) was calculated using MSCT (Somatrom Volum Zoom Siemens AG, Erlhagen, Germany). The heart was scanned during a period of 20 to 30 seconds with a distance of 3 mm between each slice. Tomographic imaging was triggered electrocardiographically at 60% of the RR interval and proceeded from the level of the carina to the diaphragm. This equipment is able to detect lesions at a density of at least 130 Hounsfield units and minimal area of 0.5 mm^2. Total CaSc calculations were based on measurements of total volume and area of calcified lesions, as well as mean and maximum density values. Partial CaSc was calculated for the left main coronary artery, the descending branch of the left coronary artery, the circumflex branch of the left coronary artery, and the right coronary artery. The scores then were added to calculate total CaSc. The final score was expressed in modified Agatston units (AU).[15] Coronary calcification was considered present when the CaSc was greater than 0 AU.

Bone biopsy

Bone specimens were obtained from the iliac crest. The procedure was conducted using a 7 mm inner diameter trephine that was adapted to an electrical drill (Gauthier Medical, Rochester, MN, USA). All patients were prelabeled with oral tetracycline (20 mg/kg per day for 3 days) administered over two separate periods 10 days apart. Bone fragments were submitted to the usual processing and histologic analysis. Bone histomorphometry was conducted using the semiautomatic method contained in the Osteomeasure software (Osteometrics, Inc., Atlanta, GA, USA). Histomorphometric parameters were those suggested by the American Society of Bone and Mineral Research.[16] The reference ranges used for static parameters were obtained from local control individuals,[17] whereas the dynamic parameters followed those described elsewhere.[18] Low-turnover bone disease was diagnosed when the bone-formation rate (BFR/BS) was lower than 0.04 $\mu m^3/\mu m^2$ per day in woman and lower than 0.06 $\mu m^3/\mu m^2$ per day in men, and high-turnover bone disease was diagnosed when the BFR/BS was higher than 0.10 $\mu m^3/\mu m^2$ per day in women and higher than 0.20 $\mu m^3/\mu m^2$ per day in men. These values correspond to 1 SD below or above the mean normal reference value for low- or high-turnover bone disease, respectively.

Statistical analysis

Mean and standard deviation, median, and range values or frequencies (proportions) were calculated for all variables. The distribution of CAC score was markedly skewed; as such, it was resistant to normalization by log transformation and other techniques. Therefore, the CAC score values were grouped into a dichotomous variable (i.e., presence of any calcification or total absence of CAC) for use in logistic regression. Comparisons of means and medians were done by Student's *t* test and the Mann-Whitney *U* test for normal and skewed data, respectively. Comparisons of proportions were done by chi-square analysis or by Fischer's exact test, when appropriate. Multiple logistic regression analysis was applied to assess the relation between the presence of calcification and natural logarithm (ln) of BFR/BS adjusted for age, sex, and diabetes. A $p < .05$ was considered statistically significant. All statistical analyses were performed using SPSS for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Table 1 lists the demographic, clinical, and laboratorial data of the 50 patients enrolled in the study. The patients were predominantly young males who had been followed by a

Table 1. Demographics and Clinical Characteristics ($n = 50$)

Age (years)	51 ± 11
Male (%)	34 (68%)
CKD etiology (%)	
Hypertension	20 (40%)
Diabetes	15 (30%)
Others	15 (30%)
BMI > 25 kg/m^2 (%)	27 (54%)
Creatinine clearance (mL/1.73m^2/min)	39 ± 19
Bicarbonate (mEq/L)	24 ± 3
Ionized calcium (mmol/L)	1.30 ± 0.06
Phosphorus (mg/dL)	3.8 ± 0.7
Alkaline phosphatase (U/L)	116 (37–634)
iPTH (pg/mL)	83 (26–548)
25(OH)D (ng/mL)	30.8 ± 10.1
1.25$(OH)_2$D (pg/mL)	39.9 ± 18.1

CKD = chronic kidney disease; BMI = body mass index; results in mean and standard deviation, median, and range or *n* (%).

nephrologist for a mean time of 33 months. Hypertension and diabetes were the main causes of CKD. More than half the patients were overweight/obese (54%), and none had a BMI lower than 18 kg/m^2. The mean creatinine clearance was 39 ± 19 mL/1.73 m^2 per minute. According to the CKD classification,[21] 7 patients (14%) were in stage 2, 25 (50%) in stage 3, and 18 (36%) in stage 4. Concerning bone mineral metabolism, 64% of the patients had increased levels of iPTH, 12% had hyperphosphatemia, and 6% had increased levels of alkaline phosphatase. Vitamin D deficiency and insufficiency were observed in 10% and 32% of the patients, respectively.

Bone histomorphometric parameters are shown in Table 2. Low trabecular bone volume, increased eroded and osteoclast surfaces, and low bone-formation rate (BFR/BS) were observed. No difference in BFR/BS values and in the prevalence of bone-turnover disorders were observed between diabetic and nondiabetic patients and among CKD stages (Fig. 1). Low-turnover disease was observed in 100% of the patients in stage 2, 88% in stage 3, and 78% in stage 4. There was no evidence of aluminum or iron deposits on bone surfaces.

Thirty-three (66%) patients had CAC, and their median calcium score was 89.7 (0.4–2299.3 AU). Patient distribution according to CAC score is depicted in Fig. 2. The differences between patients with and without calcification are listed in Table 3. The group with calcification was older, predominantly male, presented with a higher prevalence of diabetes, and had lower levels of ionized calcium. Concerning bone histomorphometric parameters, patients with calcification had a lower BFR/BS. Other parameters were similar between both groups. Multiple logistic regression analysis adjusted for age, sex, and diabetes showed that the presence of coronary calcification was independently associated with lower ln BFR/BS ($p = .009$, OR = 0.15, 95% CI 0.036–0.619).

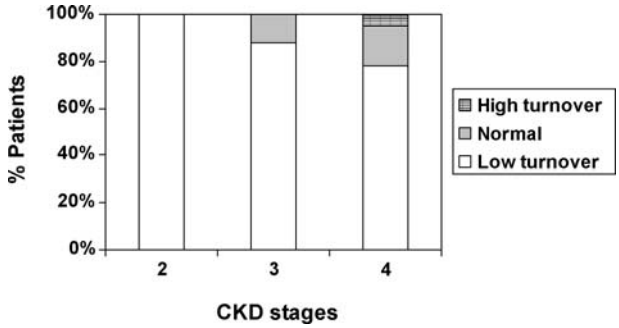

Fig. 1. Bone turnover status according CKD stages.

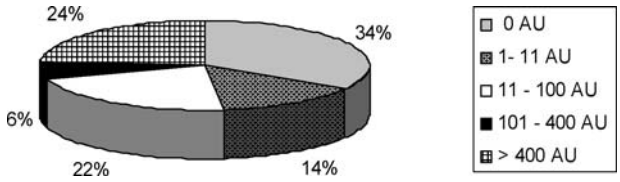

Fig. 2. Patients distribution according to CAC score.

Discussion

In this study we showed not only that coronary calcification is highly prevalent in asymptomatic CKD predialysis patients but

Table 2. Bone Histomorphometry ($n = 50$)

		Reference ranges	
		Male	Female
Structure			
BV/TV (%)	17.2 ± 5.7	24.0 ± 6.1	21.8 ± 7.2
Tb.Th (μm)	124.3 ± 26.8	127.9 ± 29.7	126.0 ± 28.8
Tb.Sp (μm)	658.3 ± 237.1	420.6 ± 124.1	498.3 ± 195.9
Tb.N (number/mm)	1.38 ± 0.38	1.89 ± 0.42	1.76 ± 0.52
Formation			
OV/BV (%)	2.29 ± 2.5	2.9 ± 2.7	1.55 ± 1.9
Ob.S/BS (%)	1.8 ± 2.2	1.2 ± 1.4	1.2 ± 3.2
O.Th (μm)	7.8 ± 3.1	11.7 ± 3.5	10.8 ± 3.2
OS/BS (%)	15.5 ± 13.6	16.1 ± 12.6	9.2 ± 8.4
BFR/BS (μm^3/μm^2/day)[a]	0.017 ± 0.02	0.13 ± 0.07	0.07 ± 0.03
MLT* (days)[a]	204.8 ± 240.5	21.3 ± 2.3	23.7 ± 2.7
Resorption			
ES/BS (%)	7.8 ± 6.4	1.75 ± 1.21	2.3 ± 2.4
Oc.S/BS (%)	0.73 ± 0.79	0.03 ± 0.11	0.03 ± 0.06
Fibrosis			
Fb.V/BV (%)	0.05 ± 0.10	0	0

BV/TV = trabecular bone volume; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Tb.N = trabecular number; OV/BV = osteoid volume; Ob.S/BS = osteoblast surface; O.Th = osteoid thickness; OS/BS = osteoid surface; BFR/BS = bone formation rate; MLT = mineralization lag time; ES/BS = eroded surface; Oc.S/BS = osteoclast surface; Fb.V/BV = fibrosis volume.

*$n = 45$. Results in mean and standard deviation values. Reference ranges from dos Reis and colleagues.[17]

[a]Reference ranges from Melsen and colleagues.[18]

Table 3. Comparison Between Patients With and Without Calcification ($n = 50$)

	Without calcification ($n = 17$)	With calcification ($n = 33$)	p
Age (years)	46 ± 12	54 ± 9	.01
Male gender (%)	47	79	.02
Diabetes (%)	18	51	.03
Creatinine clearance (mL/1.73 m^2/min)	38 ± 17	39 ± 19	.81
Ionized calcium (mmol/L)	1.32 ± 0.04	1.29 ± 0.06	.01
Phosphorus (mg/dL)	3.9 ± 0.6	3.7 ± 0.8	.49
Intact parathyroid hormone (pg/mL)	92 (33–416)	108 (26–548)	.12
Alkaline phosphatase (U/L)	95 (37–257)	122 (51–634)	.52
$1,25(OH)_2D$ (pg/mL)	38.7 ± 14.3	41.2 ± 20.5	.66
25(OH)D (ng/mL)	33.4 ± 9.9	29.4 ± 10.1	.17
Bicarbonate (mEq/L)	24 ± 4	24 ± 3	.62
BVTV (%)	18.8 ± 5.2	16.3 ± 5.8	.16
OVBV (%)	2.4 ± 1.8	2.2 ± 2.8	.20
Ob.S/BS (%)	2.00 ± 1.69	1.72 ± 2.43	.21
ES/BS (%)	8.98 ± 6.82	6.81 ± 5.86	.30
Oc.S/BS (%)	0.88 ± 0.70	0.65 ± 0.83	.10
BFR (μ^3/μ^2/day)	0.028 ± 0.021	0.012 ± 0.021	$<.001$

BV/TV = trabecular bone volume; OV/BV = osteoid volume; Ob.S/BS = osteoblast surface; ES/BS = eroded surface; Oc.S/BS = osteoclast surface BFR/BS = bone-formation rate. Results in mean and standard deviation, median, and range or *n* (%).

also, and more important, the association between coronary calcification and low bone-formation rate. Coronary calcification has been demonstrated recently to be frequent and severe in CKD patients, including those not on dialysis.[10,11,13] Traditional cardiovascular risk factors have been associated with the occurrence of vascular calcification in this population.[20,21] Accordingly, in this study, the group of patients with coronary calcification showed a significantly higher prevalence of cardiovascular traditional risk factors, such as older age, male gender, and diabetes, than those with no coronary calcification. However, it is well known that the traditional risk factors do not completely account for coronary calcification in these patients. Thus nontraditional risk factors, such as those related to bone disorders, have been proposed to contribute to the appearance and development of vascular calcification in CKD patients, particularly in those undergoing dialysis.[22,23] Renal osteodystrophy is a common complication of CKD,[24] and hyperparathyroid bone disease has been described as the most frequent disorder observed in dialysis patients.[25–27] Nevertheless, data regarding bone disorders in predialysis patients are scarce and conflicting. While earlier reports pointed out a high prevalence of high-turnover bone disease,[28,29] more recent reports have described low-turnover bone disease as the most prevalent disorder in CKD stage 5 patients just before entering dialysis.[28–31] In this study, which included patients in CKD stages 2 to 4, low-turnover bone disease with a markedly reduction in bone-formation rate was observed in most patients. The finding of low bone turnover in 88% of patients is surprising and could be due to the reference values for the dynamic parameters[18] adopted in our laboratory. Of note, there are few laboratories in the world that have published the reference values for dynamic parameters of bone histomorphometry because they have to be obtained from bone samples in vivo. Although there are some variability in the lower BFR/BS values from those laboratories, most are not lower than 0.04 $\mu m^3/\mu m^2$ per day. Therefore, the lower BFR/BS values seen in this study are in accordance with the diagnosis of low-turnover bone disease. It is important to point out that the studied population consisted of asymptomatic and well-nourished patients not receiving vitamin D or phosphate binders

Several factors, such as diabetes, old age, and aluminum intoxication, have been described to explain the reduction in bone-formation rate in CKD patients. Moreover, in CKD patients not on dialysis, skeletal resistance to PTH may play an important role.[27] Apparently, PTH receptors in osteoblasts are downregulated by several still unknown uremic inhibitors.[32,33] Accordingly, recent data confirmed that higher levels of PTH would be necessary to stimulate and increase bone turnover in this population.[34,35] However, it seems that other factors may be implicated in the decrease in bone remodeling because in this study, despite having mean PTH levels higher than those proposed by the K/DOQI guidelines, most patients had low bone turnover, and no patient from the studied population had aluminum intoxication.

Results from experimental studies have suggested that the metabolic syndrome might be associated with significant reductions in skeletal bone-forming/modeling units and with a tendency for a reduced mineralizing bone surface and osteoblast number.[36] Of note, superimposing ablative CKD in that model resulted in low-turnover bone disease. In this study, a high prevalence of hypertension, diabetes, overweight/obesity, and dyslipidemia was observed. Such conditions, considered to be part of the metabolic syndrome, could have contributed to make this population more suceptible to decreased bone remodeling.

Recently, a novel hormonal cascade involving *FGF23* and *Klotho* has been identified as an important factor in the regulation of phosphate, vitamin D homeostasis, and bone mineralization.[37] An in vitro study showed that *FGF23* overexpression inhibits osteoblast differentiation and matrix

mineralization, suggesting that *FGF23* could inhibit bone formation independently of its effects on phosphurus homeostasis.[38] Additionally, *FGF23*-null mice have soft tissue calcifications, severe growth retardation, and abnormalities of bone mineralization.[39] Other potential mechanisms of low bone-formation rate in CKD include decreased circulating levels of bone anabolic factors such as insulin-like growth factor 1 (IGF-1)[40] and bone morphogenetic protein 7 (BMP-7).[41] In fact, IGF-1 levels directly correlate with bone formation in dialysis patients, independent of PTH,[42] and BMP-7 is an osteoblast growth and differentiation factor that seems to be reduced in CKD.[43] Unfortunately, those factors were not measured in this study, preventing further conclusions regarding their specific role in the decreased bone turnover observed in this cohort.

Notably, despite the decreased bone-formation rate found in this study, bone reabsorption rate was significantly elevated, resulting in severe loss of bone and leading to a remarkably low trabecular bone volume, a condition that may contribute to the development to CAC. Epidemiologic studies in elderly and postmenopausal women have demonstrated that patients with osteoporosis have increased CAC.[44,45] More important, in postmenopausal women, the loss of bone was associated with progression of vascular calcification.[46,47] Similarly, Braun and colleagues documented an inverse correlation between CAC and bone mineral density in hemodialysis patients.[48] More recently, London and colleagues demonstrated that patients on hemodialysis, with the lowest bone-formation rate and decreased osteoblast surfaces, had the greatest degree of peripheral artery calcification evaluated by ultrasound.[23] In a prospective study performed by our group, the improvement in bone-formation rate in patients with low-turnover bone disease on hemodialysis therapy was associated with lower progression of CAC.[22]

A similar association between CAC and low bone-formation rate in predialysis patients was observed in this study. It has been suggested that the decreased bone-formation rate leads to an inability of bone to buffer the calcium overload, predisposing to metastatic calcification.[49] Unexpectedly, in this study, patients with calcification showed lower levels of ionized calcium than those with no calcification. It has been suggested that the lower levels of calcium in patients with vascular calcification could be due to the preciptation of calcium salts in the vessel walls or a consequence of vitamin D insufficiency or deficiency. Actually, this latter condition could be implicated in the decrease in bone remodeling in patients with calcification.[50] However, in this study there was no diference in either vitamin D levels or the prevalence of vitamin D insufficiency and deficiency between patients with and without calcification. Of note, when patients were divided in two groups according vitamin D profile (insuficient versus suficient) no differences were observed for BFR and CAC between groups (data not shown).

Although our study included a small number of patients and did not allow us to determine the natural history of bone disease in predialysis patients or to establish the cause-effect relationship between vascular calcification and bone disorders, to the best of our knowledge, this is the first study that evaluates the association between vascular calcification and bone histomorphometric parameters in CKD patients not on dialysis.

In conclusion, our results provide evidence that low bone-formation rate constitutes another nontraditional risk factor for cardiovascular disease in CKD patients. Efforts should be taken to better understand mineral and bone metabolism to avoid conditions that may impair bone remodeling in order to reduce or even prevent vascular calcification in patients in earlier stages of CKD.

Disclosures

No company provided funding for this trial. The investigators are solely responsible for the design, conduct, analysis, and publication of the trial. There were no restrictions on publication, and all data were maintained and analyzed solely by the authors. The authors state that they have no conflicts of interest.

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