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The link between bone and coronary calcifications in CKD-5 patients on haemodialysis

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Abstract

Background. Vascular calcifications are frequent in Stage 5 chronic kidney disease (CKD-5) patients receiving haemodialysis. The current study was designed to evaluate the associations between bone turnover/volume and coronary artery calcifications (CAC).

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Methods. In 207 CKD-5 patients, bone biopsies, multislice computed tomography of the coronary arteries and blood drawings for relevant biochemical parameters were done. The large number of CKD-5 patients enrolled allowed separate evaluation of patients with CAC *versus* patients without CAC and adjustment for traditional and non-traditional risk factors for CAC.

Results. When all patients were analysed, associations were found between CAC and bone turnover, bone volume, age, gender and dialysis vintage. When only patients with CAC were included, there was a U-shaped relationship between CAC and bone turnover, whilst the association with bone volume was lost. In these patients, the relationship of CAC with age, gender and dialysis vintage remained.

Conclusions. Beyond the non-modifiable risk factors of age, gender and dialysis vintage, these data show that bone abnormalities of renal osteodystrophy amenable to treatment should be considered in the management of patients with CAC.

Keywords: bone turnover; bone volume; coronary calcifications; dialysis; renal osteodystrophy

Introduction

Abnormalities in bone and mineral metabolism are common complications in chronic kidney disease (CKD) patients [1]. Recent evidence shows that these abnormalities are associated with vascular calcifications, cardiovascular disease (CVD) and decreased quality of life [2–6]. In Stage 5 CKD (CKD-5) patients on dialysis, vascular calcifications occur more frequently and progress more rapidly than in the general population [7,8]. Moreover, the presence of vascular calcifications has been reported as an independent risk factor for mortality [9]. In addition to traditional risk factors of CVD, chronic kidney disease–mineral bone disorder (CKD-MBD) has been suggested as a non-traditional risk factor to explain the high rates of CVD in CKD patients [10–12].

It has been demonstrated that vascular calcification scores are associated with low bone turnover [5] and low bone volume [6]. No information is available on differences between CKD-5 patients with and without coronary artery calcifications (CAC) and evaluation of CKD-5 patients with CAC stratified according to turnover states. This appears clinically important because experience in the past demonstrated associations of vascular calcifications in patients with hyperparathyroidism [13,14], whilst more recent data demonstrate associations with low bone turnover [5].

The present study was designed to investigate in CKD-5 patients the relationship between CAC (evaluated by multislice computed tomography [MSCT]) and parameters of bone turnover and bone volume (evaluated by histomorphometry) with adjustment for a large number of risk factors of vascular calcifications. The number of patients was sufficiently large (i) to allow comparison of patients with and without CAC and (ii) to stratify patients with CAC by low, normal and high bone turnover to evaluate the role of bone parameters and of known cardiovascular risk factors for their associations with more extensive CAC.

Materials and methods

Patients and study design

For this cross-sectional study, 853 CKD-5 patients receiving haemodialysis (HD) in eight HD centres in Izmir, Turkey were screened and 207 patients agreed to undergo bone biopsy, determination of CAC score by MSCT and blood drawing for research purposes. These 207 patients are representative of the overall patient population treated in these units with respect to age, gender, presence of diabetes mellitus and range of parathyroid hormone (PTH) levels. All patients were receiving thrice weekly conventional HD treatment using high-flux (70%) and low-flux (30%) dialysis membranes and a dialysate calcium of 1.62 mmol/L. Causes for the development of CKD-5 requiring dialysis therapy were diabetes mellitus (26%), hypertension (15%), glomerular disease (26%) and unknown (33%). The study was conducted according to the Declaration of Helsinki and the protocol was reviewed and approved by the institutional review boards of all participating institutions. All patients gave informed consent.

Inclusion criteria were age ≥ 18 years, maintenance HD thrice weekly (12 h/week), naïve or on steady dose of vitamin D analogues for at least 6 months and willingness and mental competence to sign informed consent for bone biopsy, MSCT and blood drawing.

Exclusion criteria were being scheduled for live donor renal transplantation; pregnancy or lactation; serum calcium ≥ 10.5 mg/dL; history of parathyroidectomy; use of calcimimetics; cardiac arrhythmia (hinders ECG gating of MSCT); prior coronary angioplasty, stent placement or prior coronary bypass grafting (causing artefacts on MSCT scans) and life-threatening co-morbid conditions such as malignancy, active infection, end-stage cardiac/pulmonary/hepatic disease.

Hypertension was defined as blood pressure ≥ 140/90 mmHg. Blood pressure measurements were made manually using an Erka sphygmomanometer after a 5-min rest just before and after dialysis session.

CVD history was defined by documentation of chronic stable angina, chronic unstable angina, peripheral artery disease, peripheral artery angioplasty or stent, carotid artery disease, carotid artery stenosis by ultrasound or angiography, carotid endarterectomy, prior ischaemic cerebrovascular accident and abdominal artery disease.

For each patient, bone biopsy and MSCT were performed within a time period of 3 months.

Mineralized bone histology and bone histomorphometry

For double labelling of bone, patients received oral tetracycline hydrochloride 500 mg twice daily for 2 days followed by a 10-day tetracycline-free interval and another course of tetracycline hydrochloride at the same dosage for 4 days. Posterior iliac crest bone biopsies were performed after an additional 4 days (bone samples = 0.3 cm diameter × 2 cm length). Iliac crest bone samples were fixed with ethanol at room temperature, dehydrated and embedded in methyl methacrylate as described previously [15]. Serial sections of 3- and 7-μm thickness were cut with a microtome (Model HM360, Microm, Walldorf, Germany) equipped with a carbide-edged knife. Sections were stained with the modified Masson–Goldner trichrome stain [16], the aurintricarboxylic acid stain [17] and solochrome azurine stain [18]. Unstained sections were prepared for phase-contrast and fluorescence light microscopy. Bone histomorphometry for static and dynamic parameters of bone structure, formation and resorption was done at a magnification ×200 using the Osteoplan II system (C. Zeiss, New York, NY). All bone samples were processed and analysed at the Bone Diagnostic and Research Laboratory, University of Kentucky, Lexington, KY, USA. All measured histomorphometric parameters were in compliance with the recommendations of the nomenclature committee of the American Society of Bone and Mineral Research [19].

The classification of 'low', 'normal' and 'high' bone turnover was based on our normative database [20–22]. The outcome group 'low' bone turnover was defined as activation frequency (Ac.f.) <0.49 $year^{-1}$ and/or bone formation rate/bone surface (BFR/BS) <1.8 mm^3/cm^2/year. The outcome group 'normal' bone turnover was defined as Ac.f. 0.49–0.72 $year^{-1}$ and/or BFR/BS 1.8–3.9 mm^3/cm^2/year. The outcome group 'high' bone turnover

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was defined as Ac.f. >0.72 year^{-1} and/or BFR/BS >3.9 mm^3/cm^2/year. Bone volume (BV/TV) was classified as "low" (<16.8%), "normal" (16.8–22.9%), and "high" (>22.9%).

Coronary artery calcification score

CAC score was measured by a dual-score 64-slice MSCT scanner (Aquilion 16, Toshiba Medical Systems Corporation, Tokyo, Japan) using a calcium-scoring programme (Terarecon 3.4.2.11, San Mateo, CA, USA). MSCT scans were performed with quad-slice technique. All radiological examinations were performed between two dialysis sessions at the same institution by using the same device and assessed by the same operator. Scan slices of 3.0 mm thickness were acquired under the following conditions: 250 mA of tube current 62 mAs effective. Images were obtained during a single breath-hold of 12–15 s. Data obtained during the diastolic phase of the cardiac cycle were used for image reconstruction with the use of ECG monitoring. Threshold calcium determination was set using a density of at least 130 Hounsfield units. The CAC score was calculated by summing the calcification score in the left main coronary artery, left anterior descending artery, left circumflex and right coronary artery. The CAC score was evaluated according to the originally described method of Agatston *et al*. [23].

Laboratory measurements

In all patients, blood samples were obtained using uniform techniques in all centres under fasting conditions immediately before their scheduled dialysis sessions. Blood chemistry including high-sensitivity C-reactive protein (hs-CRP) were done by using standardized and automated techniques (Architect C8000 auto-analyser, Abbott, Chicago, IL, USA) in the same laboratory keeping external quality control programmes. Intact PTH (iPTH) level was measured by radioimmunometric assay (Scantibodies Inc., Santee, CA, USA): normal range is 14–66 pg/mL; intra-assay and inter-assay coefficients of variation are <5 and <7%, respectively.

Statistical analysis

Continuous variables are expressed as mean ± SD and frequency counts as percentages. P-values ≤ 0.05 were considered to be statistically significant. Between-group comparisons for continuous variables were performed using ANOVA; χ^2 or Fisher's exact test was used for categorical variables. Spearman correlation coefficients were used to assess the bivariate relationships between CAC scores and demographic, laboratory or bone parameters. Multiple ordinal logistic (proportional odds) regression was used to determine independent associations of predictor variables with CAC score categories (1–100, 101–400 and >400). Polytomous response logistic regression was used in cases where the proportional odds assumption did not hold. The regression models considered had different primary predictor variables (Ac.f. or BFR/BS or bone volume/tissue volume [BV/TV]). Independent variables for inclusion were selected using a backward stepwise algorithm with P ≤ 0.10 to enter the model and P < 0.05 to remain in the model. The starting models contained one of the primary predictor variables and smoking, age, gender, HD duration, presence of diabetes mellitus, vitamin D usage and all two-way interaction terms involving the primary predictor.

All statistical calculations were performed with SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) and R version 2.7.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of the study population

Demographic and laboratory parameters of the study population (N = 207) categorized by absence or presence of CAC are summarized in Table 1. Between patients with and without CAC, there were no statistically significant differences in vitamin D treatment, dose of calcium-based binder hypertension and in blood levels of calcium, phosphorus, iPTH, triglycerides and cholesterol. There were more smokers among the patients with CAC, but this difference did not reach statistical difference. However, patients with CAC were older, had higher serum levels of C-reactive protein (hs-CRP), were more likely to be male, had diabetes mellitus and had a history of coronary artery disease.

In all patients, low bone turnover was found in 71%, normal bone turnover in 17% and high bone turnover in 12% (determined by Ac.f. and BFR/BS). BV/TV was low in 42% of patients, normal in 32% and high in 26%. None of the biopsy specimen stained positive for aluminium.

Mean CAC score was 444 ± 877 [range of 0–6106]. In patients with CAC (N = 143), histomorphometric parameters of bone showed significantly lower BFR/BS and lower Ac.f, although the latter did not reach statistical significance

Table 1. Demographic and biochemical characteristics of the study population stratified according to CAC = 0 or CAC > 0

	CAC = 0, N = 64	CAC > 0, N = 143	P-value
Age (years)	47 ± 15	63 ± 12	<0.0001
Gender (male, %)	41	57	0.05
HD duration (months)	46 ± 33	53 ± 41	0.40
Diabetes (%)	6	26	0.002
CVD (%)	5	27	0.0001
Hypertension (%)	6	14	0.16
Smoking (%)	23	38	0.06
Elementary calcium dosage (g/day)	1.035 ± 0.716	0.940 ± 0.545	0.92
Vitamin D treatment (μg/week)	3.75 ± 1.50	3.45 ± 1.29	0.67
25(OH) vitamin D (ng/mL)	41.1 ± 3.3	38.6 ± 1.9	0.71
Albumin (g/dL)	4.0 ± 0.3	3.9 ± 0.3	0.05
Calcium (mg/dL)	9.2 ± 0.9	9.2 ± 0.9	0.88
Phosphorus (mg/dL)	5.2 ± 1.6	5.0 ± 1.5	0.72
iPTH (pg/mL)	187 ± 188	220 ± 265	0.74
Total cholesterol (mg/dL)	181 ± 36	185 ± 47	0.68
Triglyceride (mg/dL)	147 ± 75	167 ± 109	0.42
Hs-CRP (mg/dL)	0.97 ± 1.85	1.30 ± 2.02	0.03

Between-group comparisons for continuous variables were performed using ANOVA; χ^2 or Fisher's exact test was used for categorical variables. HD, haemodialysis; CVD, cardiovascular disease; iPTH, intact parathyroid hormone; hs-CRP, high-sensitivity C-reactive protein.

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Table 2. Static and dynamic bone parameters stratified according to CAC = 0 *versus* CAC > 0

	CAC = 0, *N* = 64	CAC > 0, *N* = 143	P-value
ObS/BS (%)	1.54 ± 2.15	1.74 ± 3.43	0.26
OcS/BS (%)	1.49 ± 1.64	1.19 ± 1.26	0.25
BFR/BS (mm^3/cm^2/year)	1.85 ± 1.71	1.53 ± 2.27	0.05
Ac.f. ($year^{-1}$)	0.39 ± 0.36	0.33 ± 0.45	0.07
BV/TV (%)	20.32 ± 8.07	19.05 ± 7.31	0.27

Between-group comparisons for continuous variables were performed using ANOVA. CAC score, coronary artery calcification score; iPTH, intact parathyroid hormone; ObS/BS, osteoblasts/bone surface; OcS/BS, osteoclasts/bone surface; BFR/BS, bone formation rate/bone surface; Ac.f., activation frequency; BV/TV, bone volume/tissue volume.

(Table 2). Because of these differences, patients with CAC were evaluated separately from patients without CAC.

Among patients with CAC, increasing CAC scores were associated with older age, male gender, longer HD vintage and lower albumin (Table 3). It is of note that there were no statistically significant differences between CAC score groups in smoking, diabetes mellitus, history of coronary artery disease, hypertension and blood levels of calcium, phosphorus, iPTH, triglycerides, total cholesterol and hs-CRP. Histomorphometric parameters of bone formation, resorption and turnover were not different between different CAC score groups (Table 4).

Associations between bone and coronary artery calcification in patients with and without CAC

Ordinal logistic regression analysis revealed that age (P < 0.001), dialysis vintage (P = 0.039) and bone turnover measured by Ac.f. (P = 0.026) or BFR/BS (P = 0.013) were significantly and *positively* correlated with CAC score.

When the histomorphometric parameter cancellous BV/TV was entered into the model instead of turnover parameters, lower BV/TV was associated with higher CAC score (P = 0.036). The presence of diabetes mellitus (P = 0.02) and male gender (P = 0.004) were also associated with higher CAC score groups in addition to older age (P = 0.001) and longer dialysis vintage (P = 0.044). There was an interaction between BV/TV and age (P = 0.044): more advanced age increased the effect of lower bone volume on CAC score.

Associations between bone and coronary artery calcification in patients with CAC

For analysis of bone turnover, patients with CAC were analysed after stratification into low, normal and high bone turnover groups. In patients with low bone turnover, there was a negative association between Ac.f. and CAC scores (P = 0.03) and BFR/BS and CAC scores (P = 0.01). This relationship was not found in patients with normal bone turnover (P = 0.46 and P = 0.38, respectively), whilst in patients with high bone turnover, a positive association was seen (P = 0.03 and P = 0.01, respectively).

For analysis of cancellous bone volume, BV/TV was entered as a continuous variable into linear regression models. No relationship between BV/TV and CAC scores could be identified (P = 0.98), whilst age (P = 0.001), dialysis vintage (P = 0.0047) and male gender (P = 0.044) were associated with CAC.

Discussion

The presented data in 207 CKD-5 patients on HD adjusted for traditional risk factors of vascular calcifications demonstrate intricate associations between bone turnover, bone volume and coronary calcifications. The strength of the

Table 3. Demographic and biochemical characteristics of the study population stratified according to CAC score group

	CAC score			
	1–100, *N* = 47	101–400, *N* = 39	>400, *N* = 57	P-value
Age (years)	60 ± 12	60 ± 12	65 ± 10	0.04
Gender (male, %)	38	72	61	0.005
HD duration (months)	38 ± 29	61 ± 43	61 ± 45	0.007
Diabetes (%)	22	26	29	0.73
CVD (%)	22	29	30	0.66
Hypertension (%)	13	13	16	0.65
Smoking (%)	30	41	42	0.39
Elementary calcium dosage (g/day)	0.949 ± 0.521	1.030 ± 0.554	0.860 ± 0.560	0.11
Vitamin D treatment (μg/week)	4.0 ± 1.73	3.0 ± 0.01	3.33 ± 1.37	0.11
25(OH) vitamin D (ng/mL)	34.5 ± 2.9	42.1 ± 3.7	39.2 ± 3.4	0.30
Albumin (g/dL)	4.0 ± 0.3	3.8 ± 0.2	3.8 ± 0.3	0.01
Calcium (mg/dL)	9.0 ± 0.6	9.1 ± 0.7	9.4 ± 1.2	0.09
Phosphorus (mg/dL)	4.8 ± 1.4	5.1 ± 1.5	5.2 ± 1.6	0.40
iPTH (pg/mL)	174 ± 173	229 ± 265	249 ± 320	0.34
Total cholesterol (mg/dL)	188 ± 46	176 ± 47	187 ± 46	0.43
Triglyceride (mg/dL)	178 ± 139	158 ± 81	164 ± 97	0.68
Hs-CRP (mg/dL)	1.08 ± 1.74	1.58 ± 2.41	1.29 ± 1.95	0.52

Between-group comparisons for continuous variables were performed using ANOVA; χ^2 or Fisher's exact test was used for categorical variables. HD, haemodialysis; CVD, cardiovascular disease; iPTH, intact parathyroid hormone; hs-CRP, high-sensitivity C-reactive protein.

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Table 4. Static and dynamic bone parameters stratified according to CAC score group

	CAC score			
	1–100, $N = 47$	101–400, $N = 39$	>400, $N = 57$	P-value
ObS/BS (%)	1.46 ± 1.95	1.94 ± 3.85	1.84 ± 4.07	0.79
OcS/BS (%)	1.25 ± 1.36	1.33 ± 1.31	1.05 ± 1.13	0.54
BFR/BS (mm^3/cm^2/year)	1.40 ± 1.44	2.82 ± 2.10	2.37 ± 3.63	0.32
Ac.f. ($year^{-1}$)	0.31 ± 0.31	0.62 ± 0.47	0.49 ± 0.65	0.21
BV/TV (%)	19.58 ± 8.50	17.81 ± 5.92	19.45 ± 7.14	0.47

Between-group comparisons for continuous variables were performed using ANOVA. CAC, score coronary artery calcification score; iPTH, intact parathyroid hormone; OBS/BS, osteoblasts/bone surface; OCS/BS, osteoclasts/bone surface; BFR/BS, bone formation rate/bone surface; Ac.f., activation frequency; BV/TV, bone volume/tissue volume.

current study comes from the large number of HD patients which allows adjusting for many traditional risk factors of vascular calcifications such as age, gender, diabetes mellitus, smoking, hypertension, serum lipids, history of CVD and hs-CRP. The high number of patients also allowed us to separately analyse patients with and without CAC and to further evaluate associations between degree of calcifications and directions of changes in bone turnover in patients with CAC.

There were significant demographic differences between our patients with and without CAC, justifying our approach to stratify analysis based on the presence or absence of CAC. This approach is supported by the observation that patients entering dialysis without CAC did not develop CAC during 30 months, whilst those who present with CAC show almost invariably progression of CAC [24].

When analysing all patients together, that is with and without CAC, we found a positive correlation between bone turnover and CAC. This correlation was, however, not found when patients without CAC were excluded from the analysis. Analyses using linear regression do not account for the different clinical presentations of patients with low, normal and high bone turnover. Clinical experience in the past teaches an association between extraosseous calcifications and hyperparathyroidism [14,25,26] and reversal of extraosseous calcifications with reduction of bone turnover after parathyroidectomy [27–29]. In contrast, an association between low bone turnover and vascular calcifications was described in recent years [5]. Therefore, we have analysed our patient cohort separately for patients with low, normal and high bone turnover. Our results in patients with CAC reveal that low bone turnover showed a negative correlation with CAC, whilst high bone turnover correlated positively. No association was found in patients with normal bone turnover. These data call for consideration of the level of bone turnover in the evaluation of patients with CAC. Although the choice of a higher dialysate calcium concentration used in our study population than that in the US could have contributed to the relatively high prevalence of low bone turnover, a longitudinal study evaluating changes in the pattern of different bone turnover states reported a significantly increased prevalence of low bone turnover in dialysis patients observed between 1995 (<15%) and 2001 (>45%), highlighting the importance of recognizing oversuppression of bone turnover and related complications as entities with great clinical relevance [30].

Analysing all studied patients, we confirm the finding of an interaction between cancellous bone volume and age in their associations with CAC [6]. Even though it is known that advanced age is associated with cardiovascular calcifications in the general population [31], the observed interactions should prompt nephrologists to be sensitive to the problem in the dialysis population which, over recent years, has shown a trend to include more advanced age groups. Whilst the observed independent associations of dialysis vintage on CAC in addition to the known factors age, diabetes mellitus and male gender are all non-modifiable risk factors of CAC, cancellous bone volume could potentially be influenced by guided therapeutic endeavours. It awaits prospective studies are required to discern whether changes in cancellous bone volume are associated with alterations in CAC.

We want to acknowledge the following limitations of our study: (i) Because of the cross-sectional study design, we were not able to draw inferences on changes of CAC overtime; of course, cross-sectional studies cannot address pathogenetic mechanisms. However, the reported specific associations between bone turnover, bone volume and CAC evaluated separately in patients with and without CAC are novel and provide justification for more complex prospective studies evaluating different therapeutic interventions. (ii) Patients were recruited from several centres in Turkey, limiting generalizability of our findings to other ethnic/racial populations.

Our findings on the significance of bone turnover and volume for coronary calcifications do not imply that known traditional risk factors do not play a role in the development of vascular calcifications. One can surmise that avoidance of abnormal bone turnover and low bone volume may increase the relevance of traditional cardiovascular risk factors established in the general population.

Our study results are of great clinical importance for the practicing nephrologist. In addition to demonstrating non-modifiable predictors of CAC in HD patients such as age, HD duration, male gender and diabetes mellitus, we report bone turnover and bone volume as non-traditional risk factors that are amenable to therapeutic endeavours. Thus, there might be avenues to broaden the goal of bone man-

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agement beyond its customary horizon by appreciating the link between bone metabolism and coronary calcifications. The latter represent a major cause of morbidity and mortality in patients receiving chronic maintenance dialysis.

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