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Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification in haemodialysis patients

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Abstract

Background. Vascular calcification has detrimental consequences on chronic kidney disease (CKD) patients, yet its pathogenesis is not fully understood. Fibroblast growth factor-23 (FGF-23) is involved in the regulation of mineral metabolism which may in turn affect vascular calcifica-

tion. Data on the relationship between FGF-23 and peripheral vascular calcification, using conventional radiographs, are conflicting, and less is known about its relation to aortic calcification. We conducted this study to investigate the relationship between FGF-23 and aortic calcification in a standard haemodialysis setting.

Methods. The study included 65 haemodialysis patients (46 prevalent and 19 incident) on a three times 4-h dialysis schedule as well as 15 controls. Those with diabetes, oral anticoagulation or parathyroidectomy were excluded. Intact FGF-23, parathormone, lipids, calcium and phosphorus were measured. Aortic calcification index (ACI) was assessed by a non-contrast computerized tomography (CT) of the abdominal aorta.

Results. FGF-23 levels were higher among haemodialysis patients (4681.3 ± 3906.1 pg/mL) compared to controls (98.2 ± 51.9 pg/mL), P = 0.005. ACI was higher in haemodialysis patients (14.1 ± 12) than controls (3.2 ± 3.6), P = 0.009. FGF-23 (P < 0.0001) and systolic blood pressure (BP) (P < 0.0001) were independently related to ACI in stepwise multiple regression analysis of pooled analysis of haemodialysis patients, R^2 = 0.476; in subgroup analysis, the independent factors relating to ACI among prevalent dialysis patients were systolic BP (P < 0.0001), FGF-23 (P = 0.002) and age (P = 0.012), R^2=0.48; whereas in incident patients, only FGF-23 was associated with ACI (P = 0.007), R^2 = 0.37.

Conclusions. In haemodialysis patients, FGF-23 and ACI were significantly increased, and FGF-23 was independently associated with aortic calcification.

Keywords: CKD–BMD; FGF-23; haemodialysis; vascular calcification

Introduction

Factors affecting vascular calcification, a predictor of morbidity and mortality in dialysis patients, are not fully uncovered [1]. Fibroblast growth factor-23 (FGF-23), a phosphaturic factor produced by osteoblasts, has emerged in the past decade as a major regulator of mineral metabolism in health and disease. Under physiological conditions, it acts as a circulating phosphaturic factor through suppression of proximal tubular Na/Pi-2a and Na/Pi-2c cotransporters [2–5]. In humans, activating gene mutations are associated with hypophosphataemic rickets, whereas inactivating mutations result in familial tumoral calcinosis [6,7].

FGF-23-null mice suffer severe vascular calcification in conjunction with hyperphosphataemia and increased mortality [8] suggesting that FGF-23 may be protective from vascular calcifications. Recent observations have suggested that many of the biological effects of FGF-23 are mediated by its cofactor, klotho. Klotho knockout mice display features similar to FGF-23 knockout mice with hyperphosphataemia, ectopic calcifications and shortened lifespan [3,8]. FGF-23 was found to be consistently elevated, and renal klotho mRNA expression reduced in patients with chronic kidney disease (CKD) [3,9,10].

Contrary to the impression created by the previously mentioned findings, the elevated FGF-23 was found to be associated with mortality in haemodialysis patients [9,10]. Its relation to vascular calcification in CKD patients is more ambiguous; one recent study reported an independent positive correlation between FGF-23 and peripheral vascular calcification [11], whereas another study reported a negative association [12]. Even less clear is the relation of FGF-23 to aortic calcification which, unlike peripheral calcification, is associated with coronary artery calcification. Both aortic and peripheral vascular calcifications were associated with mortality in CKD [13–15]. Nonetheless, previous studies have measured C-terminal FGF-23, used conventional radiography for evaluation of vascular calcification and included patients with possible confounding factors that may alter vascular calcification patterns and interact with FGF-23, *viz*. diabetes and warfarin intake, as well as study exceptional patients on long 8-h haemodialysis [11,12].

The aim of this study is to investigate the relation between intact FGF-23 and aortic calcification in a standard three times 4-h incident and prevalent haemodialysis non-diabetic patients not receiving warfarin. A non-contrast computerized tomography (CT) of the abdominal aorta was used for the detection of vascular calcification; this technique is more quantitative than conventional X-rays and more readily available than electron beam-computed tomography (EBCT) and multislice tomography (MSCT) used for measuring coronary artery calcification [1,14–17].

Materials and methods

Eighty subjects attending our university hospital were recruited. Eligible subjects were non-diabetic, were not on oral anticoagulant therapy and had no history of parathyroidectomy or parathyroid ablation. This included all eligible incident and prevalent chronic haemodialysis patients at our centre and 15 eligible control subjects. Prevalent patients were on dialysis for at least 6 months prior to inclusion, and incident patients were on dialysis for <2 weeks. We recruited the 46 eligible prevalent dialysis patients receiving 4-h sessions, three times weekly, using low-flux polysulphone dialysers and dialysate calcium of 1.5 mmol/L. Nineteen CKD patients indicated for initiation of regular chronic dialysis were initially treated in our hospital and recruited in the study before referral to other dialysis centres for continuation of treatment. Fifteen consecutive inpatients undergoing non-contrast abdominal CT scan served as controls. The controls had estimated glomerular filtration rate (eGFR) >60 mL/min and had no proteinuria or active urinary sediment in urinalysis as well as apparently normal kidneys in the CT scans. They were all inpatients hospitalized for other diseases. The patients were informed about the study procedures and consented to participation, and local research ethics committee approval was obtained.

Blood samples were drawn and stored at −70°C. Serum levels of intact FGF-23 molecule were determined using a two-site (NH2-terminal/C-terminal) enzyme-linked immunosorbent assay (Immutopics, CA, USA). Intact parathormone levels were determined by enzyme-amplified sensitivity immunoassay (Roche Diagnostics, IN, USA).

Aortic calcification index (ACI) was determined semi-quantitatively using non-contrast axial CT scans of the abdominal aorta (Figure 1) by a method used by others [16–18] and summarized as follows: the cross section of each of 10 slices of the abdominal aorta was divided into 12 sectors. The number of sectors showing calcification in each slice was counted, and the total number of calcified sectors in the 10 slices was added up. All detectable calcifications ≥100 HU were documented, and the sum was then divided by 120 and multiplied by 100 to be expressed as a percentage. ACI determination was performed by a single investigator blinded to all other

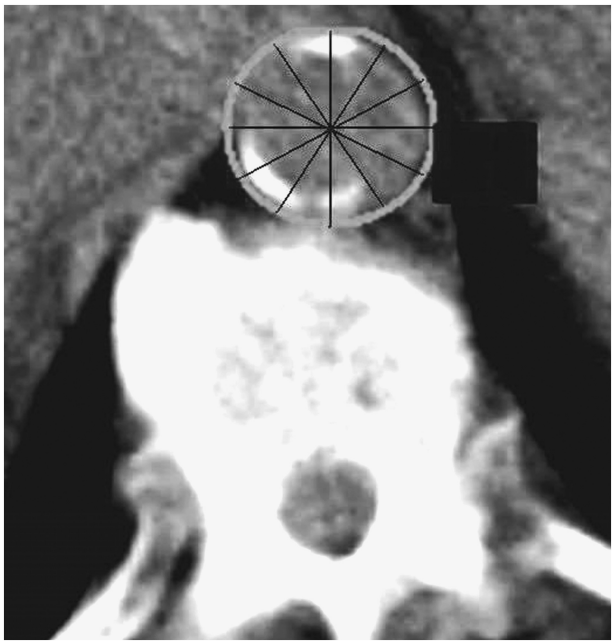

Fig. 1. ACI measurement (score 6/12 in this cut).

data of the study with an intra-observer variability of 3.7%, pretty similar to a previous report using the same technique [16].

We recorded medical history and prescriptions over past 3 months. Body weight and mean pre-dialysis blood pressure recordings were reported; for prevalent patients, we reported the mean recordings over the month preceding recruitment in the study. Pre-dialysis blood tests were performed for serum calcium, phosphorus, creatinine, lipid profile and albumin. GFR was estimated for incident dialysis patients prior to enrolment for dialysis using the abbreviated Modification of Diet in Renal Disease (MDRD) equation [19]. Serum calcium levels were corrected for serum albumin = measured Ca level in milligram per decilitre + (4.0 − albumin level in gram per decilitre)

Statistical Package for Social Analysis (SPSS) version 7.5 was used for data analysis. Data were summarized as mean, standard deviation and median. Comparison between groups was performed by Student's *t*-test; Mann–Whitney *U*-test was used for analysis of non-symmetrically distributed data. Spearman's correlation was used for bivariate analysis. Stepwise multiple regression was done to demonstrate relationships between FGF-23 and ACI with other studied factors.

Results

Pooled analysis

The demographic and clinical characteristics of study participants are presented in Table 1**.** Our control patients were hospitalized with the diagnosis of heart failure in five patients, ischaemic cerebrovascular stroke in two, complications of liver cirrhosis in two, chronic obstructive pulmonary disease complicated by chest infection in three, severe anaemia in two and severe arthritis in one. Aortic calcification index and FGF-23 were significantly higher among dialysis patients compared to controls. Only one CKD patient out of 65 had ACI of 0 (a 38-year-old male on dialysis for 24 months) *versus* eight out of 15 controls.

Table 1. Comparison of the data of dialysis patients and controls

	Haemodialysis patients	Controls	P-value
Number (*n*)	65	15	
Age	50 ± 11.5 (50; 19–73)	45.7 ± 12.7 (47; 24–64)	0.52
Sex (males)	28 (43%)	10 (66.7%)	0.75
Smokers (*n*, %)	15 (23%)	4 (26.6%)	0.72
Systolic BP (mmHg)	150 ± 20.2 (150; 90–190)	130.9 ± 18 (135; 100–160)	0.004
Diastolic BP (mmHg)	91 ± 11.3 (90; 60–110)	81.2 ± 11.5 (80; 70–100)	0.016
Calcium (mg/dL)	8.9 ± 1 (8.9; 6.2–10.8)	8.4 ± 1.1 (8.8; 7–10.5)	0.7
Phosphate (mg/dL)	6.8 ± 2.7 (6.2; 3.1–16.7)	4.1 ± 0.88 (4; 2.7–5.5)	<0.0001
Cholesterol (mg/dL)	154.9 ± 52 (146; 36–323)	160.1 ± 38 (162.2; 105–223)	0.18
Triglycerides (mg/dL)	127 ± 63.9 (102; 50–349)	108.7 ± 43.1 (102; 41–201)	0.52
CaCO3[a]	46 (70.7%)	0	<0.0001
Alphacalcidol[a]	19 (29.2%)	0	<0.0001
Sevelamer, cinacalcet[a]	0	0	
Erythropoietin[a]	35 (53.8%)	0	<0.0001
Albumin (g/dL)	3.4 ± 0.6 (3.4; 1.9–4.6)	3.6 ± 0.6 (3.2; 2.7–4.6)	0.84
Parathormone	314.7 ± 368.2 (169.4; 13.2–1900)	30.5 ± 17 (35.1; 12.6–66.6)	0.037
FGF-23	4681.3 ± 3906.1 (4058; 229–13 256.7)	98.2 ± 51.9 (96.1; 28.3–197.8)	0.005
ACI (%)	14.1 ± 12 (11.7; 0–67.5)	3.2 ± 3.6 (1.7; 0–12.75)	0.009

Figures reported as mean ± SD, and median and range, respectively, in parenthesis.
[a]Number of patients using the drug within preceding 3 months.

Table 2. Stepwise multiple regression for factors associated with ACI in pooled and subgroup analysis of incident and prevalent dialysis patients

	Significance; P	β	95% CI
Pooled analysis: $R^2 = 0.476$			
FGF-23	<0.0001	0.58	0.001–0.002
Systolic BP	<0.0001	0.48	0.18–0.4
Incident: $R^2 = 0.37$			
FGF-23	0.007	0.6	0.001–0.005
Prevalent: $R^2 = 0.48$			
Systolic BP	<0.0001	0.49	0.15–0.4
FGF-23	0.002	0.38	0–0.002
Age	0.012	0.3	0.084–0.63

Bivariate analysis showed that ACI correlated in dialysis patients with each of FGF-23 P < 0.0001, $R = 0.48$; dialysis vintage P < 0.0001, $R = 0.44$; systolic blood pressure P = 0.001, $R = 0.4$; and serum cholesterol P = 0.027, $R = 0.27$. Stepwise multiple regression analysis showed that the independent parameters associated with ACI were FGF-23 and systolic blood pressure (adjusted for age, dialysis vintage, diastolic blood pressure parathormone, phosphate, triglycerides and cholesterol) (Table 2). In a separate analysis, the FGF-23 data were transformed logarithmically; the results obtained for bivariate and multivariate analyses were similar to the non-log-transformed data. Figure 2 is a scattergram of the bivariate correlation between log FGF-23 and ACI ($R = 0.48$, P < 0.0001).

FGF-23 levels were highest in the first ACI tertile, 7365.5 ± 3623.7 pg/mL, and lowest in the third ACI tertile, 2676.6 ± 3194.6 pg/mL; P = 0.0001. In bivariate analysis, FGF-23 correlated to ACI P < 0.0001, $R = 0.48$; dialysis vintage P < 0.0001, $R = 0.6$; and phosphate P < 0.0001, $R = 0.5$. In stepwise multiple regression analysis, the factors independently related to FGF-23 were phosphate [P = 0.002, 95% confidence interval (CI) 215–890] and dialysis vintage (P = 0.025, 95% CI 3.4–48); $R^2 = 0.24$ (adjusted for age, sex, calcium, parathormone, intake of calcium and alphacalcidol).

No significant correlation was found in bivariate analysis between ACI and FGF-23 among controls ($R = -0.23$, P = 0.53).

Subgroup analysis

Knowing that increased vintage is a possible risk factor for increased calcification, sub-analysis of data was performed based on dialysis vintage, *viz.* incident (<2 weeks) and prevalent (>6 months) (Table 3). This analysis was intended as a further confirmation that the relationship between FGF-23 and ACI was truly independent from dialysis vintage.

Prevalent patients were on dialysis for a mean of 44.6 ± 40.8 months. Incident patients had a mean eGFR of 9.9 ± 3.8 mL/min prior to referral for dialysis. The incident patients displayed a significantly lower ACI despite being notably older and predominantly males. On the other hand prevalent patients had higher serum cholesterol and phosphate levels and had a higher prevalence of calcium intake. FGF-23 was significantly higher in prevalent patients. ACI was significantly higher among prevalent patients than controls (P = 0.009). The differences between ACI in incident patients and controls were not significant (P = 0.57). FGF-23 levels in both subgroups were significantly higher than controls (P = 0.005).

Stepwise multiple regression analysis showed that three independent parameters correlated independently with ACI among prevalent dialysis patients: systolic blood pressure, FGF-23 and age ($R^2 = 0.48$). Among incident dialysis patients, FGF-23 correlated independently to ACI ($R^2 = 0.37$). Adjustments were made for age, parathormone, phosphate, triglycerides and cholesterol. Dialysis vintage was included in the analysis for prevalent dialysis patients and eGFR for incident patients (Table 2).

Discussion

Non-contrast abdominal CT revealed high aortic calcification indices that were independently correlated to FGF-23 levels in non-diabetic dialysis patients.

The high prevalence of calcification that we found in non-diabetic patients (98%) compared to other studies re-

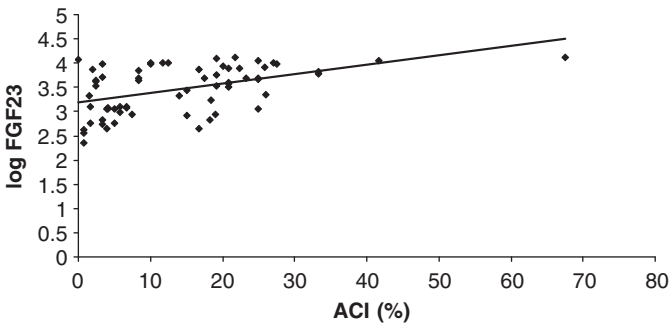

Fig. 2. Scattergram of the bivariate correlation between log FGF-23 level and aortic calcification index ($R = 0.48$, $P < 0.0001$).

Table 3. Significantly higher ACI and FGF-23 in prevalent dialysis patients

	Prevalent	Incident	P-value
Number (*n*)	46	19	
Age	46.5 ± 10.4 (47; 19–64)	59.1 ± 8.9 (59; 44–73)	<0.0001
Sex (males)	10	18	0.042
Smokers (*n*, %)	14 (30%)	1 (5%)	<0.0001
Systolic BP (mmHg)	149.2 ± 65.5 (150; 90–190)	152 ± 13 (150; 120–175)	0.66
Diastolic BP (mmHg)	90 ± 11.5 (90; 60–110)	93.6 ± 10.6 (100; 70–110)	0.48
Calcium (mg/dL)	8.9 ± 1 (8.9; 6.6–10.8)	9 ± 1 (9.2; 6.2–10.6)	0.28
Phosphate (mg/dL)	7.3 ± 2.3 (6.5; 3.7–14)	5.5 ± 3 (4.9; 3.1–16.7)	0.01
Cholesterol (mg/dL)	162.7 ± 51.5 (153; 82–323)	136 ± 49.9 (127; 36–283)	0.03
Triglycerides (mg/dL)	122 ± 56.7 (98; 50–301)	139.3 ± 79.2 (107; 65–349)	0.27
Albumin (g/dL)	3.5 ± 0.6 (3.5; 1.9–4.6)	3 ± 0.7 (3.2; 2–4.1)	0.03
CaCO3[a]	44 (95.6%)	12 (63%)	0.005
Alphacalcidol[a]	11	8	0.09
Cinacalcet, sevelamer[a]	0	0	
Erythropoietin[a]	32 (69.5%)	3 (15.8%)	0.002
Parathormone (pg/mL)	295.4 ± 314.5 (163; 13.2–1433)	361 ± 481 (170; 63–1900)	0.036
FGF-23 (pg/mL)	5959 ± 3878 (5277; 448–13 256.7)	1588 ± 1542 (1125; 229–5214.9)	0.001
ACI	17.2 ± 12.3 (18.3; 0–67.5)	6.2 ± 6.8 (4.2; 0.83–25)	0.003

Figures reported as mean ± SD, and median and range, respectively, in parenthesis.
[a]Number of patients using the drug within preceding 3 months.

porting a prevalence of 40–90% [11,12,17–21] is probably attributable to the use of computed tomography and recording calcifications at relatively low cutoff values of 100 HU, high levels of serum phosphate and dialysate calcium, and high prevalence of intake of calcium-based phosphate binders in our study. Calcification was higher in prevalent than in incident dialysis patients, as vascular calcification increases after initiation of dialysis [20,23].

The main finding of this study was that a single measurement of FGF-23 was independently correlated to aortic calcification in non-diabetic dialysis patients. Aortic calcification was shown to be associated with coronary calcification [15] and mortality [24] in CKD. The relationship between aortic calcification and FGF-23 was not demonstrated previously since preceding studies did not account for aortic calcification as a separate entity [11] or could not demonstrate a correlation with aortic calcification on lateral lumbar X-rays [12]. Why FGF-23 relates positively to vascular calcification and atherosclerosis is still largely obscure [4,11,26–28]. Effects of FGF-23 are largely mediated via its cofactor, klotho. High levels of FGF-23 as well as deficient renal klotho expression and function are observed in CKD patients. It is possible that, in the absence of klotho, the increased FGF-23 may exert pro-calcific effects via non-specific low affinity binding to its receptors [27,29,30]. Otherwise, it may be possible to view FGF-23 as a surrogate marker of other pro-calcific factors as bone alkaline phosphatase, parathormone and phosphate [11,28]. Phosphate showed no correlation with calcification in the bivariate and multivariate analyses despite high phosphate values in our study, suggesting an interaction between ACI and FGF-23 that is independent of phosphate. The correlation between phosphate and calcification in haemodialysis patients is generally not a universal finding [11,25,26].

Osteoblastic transformation and production of several bone proteins were demonstrated in the intima and media of calcified blood vessels obtained from dialysis patients [31]. Thus, FGF-23, which is also a bone-associated protein, may be a marker of the volume of the tissue producing it (i.e. the proliferating vascular osteoblasts and osteocytes). Although this hypothesis cannot be verified by our study, it is still worth testing by demonstration of FGF-23 activity and/or production in calcified vessels. This may provide an explanation for the repeatedly demonstrated reproducible correlation of 'single' FGF-23 measurements with vascular calcification, atherosclerosis and left ventricular hypertrophy under different settings [11,28,32] including our study. Indeed, a single measurement of FGF-23 predicted 1-year mortality among incident dialysis patients in one study [9] and detected 2-year mortality in prevalent patients in another study [10].

Worth mentioning are the conflicting findings of Inaba *et al*. [12] who, unlike Jean *et al*. [11], found a negative relationship between FGF-23 and peripheral calcification and, unlike our findings, found no correlation between FGF-23 and aortic calcification measured by lumbar X-rays. Both the radiological technique used and vascular ter-

ritory evaluated may explain their different results. Jean *et al*. used a calcification score which summed up calcifications on conventional radiographs from different peripheral vascular territories including popliteal, femoral and iliac vessels even including aortic calcifications as part of the score [11]; on the other hand, Inaba *et al*. evaluated hand radiographs for vascular calcification [12]. The findings of Inaba *et al*. [12] also contradict the positive association between FGF-23 and total body atherosclerosis in CKD patients using whole-body magnetic resonance angiography demonstrated by Mirza *et al*. [28]. Our technique involved CT and measured exclusively aortic calcification.

Previous studies [11,12,14,15,24,28] were vanguards that opened the way for our study. Nevertheless, this study is different in that the radiological technique used is more quantitative than conventional roentgenography and more readily available than EBCT for measuring coronary calcification [18–22]. Nonetheless, we measured intact FGF-23 rather than C-terminal, and our study population was more 'typical' on the standard three times 4-h dialysis, had higher phosphate and had higher blood pressure, making up for the shortcomings elegantly acknowledged by Jean *et al*. [11] in their landmark study on long-haemodialysis patients. We excluded diabetics and the patients on warfarin who were heavily represented in previous studies, taking into consideration that diabetes is a major risk for calcification and that the vitamin K-dependent matrix gla protein and osteocalcin may interact with FGF-23 [1,33].

The limitations of our work are the relatively small number of patients and controls, the cross-sectional nature of the study and the disregard of not integrating FGF-23 and ACI with clinical end points such as cardiovascular disease and mortality.

Conclusion

A high prevalence of aortic calcification in haemodialysis patients is detectable using non-contrast CT of the abdominal aorta. FGF-23 level correlated well to aortic calcification in pooled and subgroup analyses of incident and prevalent haemodialysis patients. Further clinical and bench studies are necessary to clarify whether FGF-23 is simply a lab indicator of—or more hopefully, a modifiable risk factor for—vascular calcification in CKD.

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Conflict of interest statement. None declared.

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Coronary artery calcification and coronary flow velocity in haemodialysis patients

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Abstract

Background. Decreased coronary flow reserve (CFR) is a marker of endothelial dysfunction, coronary artery calcification and inflammation, well-known cardiovascular risk factors in haemodialysis (HD) patients. In this study, we aimed to investigate the correlation of coronary artery calcification scores (CACS) with CFR in HD patients.

Methods. Sixty-four end-stage renal failure patients were enrolled in this study (38 males, 26 females). Thirty-nine healthy subjects (22 males, 17 females) were included in the control group. Biochemical parameters and acute-phase inflammation marker [high-sensitivity C-reactive protein (hs-CRP)] of patients were recorded before dialysis. The CACS were measured by electron beam computerized tomography method. CFR recordings were performed by transthoracic Doppler echocardiography. The relationship between CACS and CFR was evaluated.

Results. The mean CACS was 281 ± 589 and 29 patients had CACS < 10. Patients with CACS > 10 had significantly lower CFR values compared to patients with CACS < 10 (1.56 ± 0.38 *vs* 1.84 ± 0.53, P = 0.024). However, there was no difference in hs-CRP values between the groups. CFR was negatively correlated with CACS ($r = -0.276$, P = 0.030). In multiple stepwise regression analysis, CACS was found to be an independent variable for predicting CFR (P = 0.048). During a follow-up of 18 months, 10 patients had experience of cardiovascular events. Patients with CACS > 10 had significantly higher event rate [34.5% (10/29) *vs* 0% (0/24)] compared to those with CACS < 10 (P = 0.001). Patients who developed cardiovascular events had significantly higher mean CACS and lower CFR values than the remaining group (P = 0.019 and P = 0.039). All of four patients who died during follow-up were in the CFR < 2 and CACS > 10 groups.

Conclusions. CACS was associated with CFR in HD patients. However, we did not find any association of inflammation with CACS and CFR. This association between CFR and CACS might indicate two different (anatomical and functional) aspects of the common pathophysiology of the arterial system in HD patients.

Keywords: coronary artery disease; coronary flow reserve; electron beam computed tomography; haemodialysis; vascular calcification

Introduction

Cardiovascular mortality is a major cause of death in chronic kidney disease (CKD) [1,2]. In haemodialysis (HD) patients, cardiovascular complications such as endothelial dysfunction, atherosclerosis, valvular disease and left ventricular hypertrophy (LVH) are the most commonly encountered clinical challenges and the most prevalent rea-