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NEPHROLOGY - ORIGINAL PAPER

FGF-23 levels are associated with vascular calcification, but not with atherosclerosis, in hemodialysis patients

Mehmet Nuri Turan[1] · Fatih Kircelli[1] · Mustafa Yaprak[1] · Ali Riza Sisman[2] · Ozkan Gungor[1] · Selen Bayraktaroglu[3] · Mehmet Ozkahya[1] · Gulay Asci[1] · Jurgen Floege[4] · Ercan Ok[1]

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Abstract
Purpose High fibroblast growth factor-23 (FGF-23) levels are associated with mortality and cardiovascular events in patients with chronic kidney disease. The aim of this cross-sectional study was to investigate the relationship between plasma FGF-23 levels and coronary artery calcification and carotid artery intima-media thickness (CA-IMT) in hemodialysis (HD) patients.
Methods In this cross-sectional study, plasma intact FGF-23 levels were measured in 229 patients who underwent coronary artery calcification scores (CACs) determined by multi-slice computerized tomography and CA-IMT assessed by using high-resolution color Doppler ultrasonography.
Results Median FGF-23 was 53.5 pg/ml (IQR 30.8–249.5). Median CACs was 98 (IQR 0–531), and the frequency of patients with severe calcification (CACs > 400) was 28.8 %; 27.5 % of cases had no calcification. Mean CA-IMT was 0.78 ± 0.20 mm, and the presence of carotid plaques was 51 % with a mean length 2.1 mm. FGF-23 level was positively correlated with serum calcium ($r = 0.337$, $p < 0.001$), phosphate ($r = 0.397$, $p < 0.001$) and CACs ($r = 0.218$, $p = 0.001$). Neither CA-IMT nor the presence of carotid artery plaques correlated with FGF-23 levels. In adjusted ordinal regression analysis, FGF-23 level was an independent predictor for severe CACs together with age, gender, presence of diabetes, time on dialysis and CA-IMT (model $r^2 = 0.44$, $p < 0.001$). As a novel finding, the mean CACs was markedly higher in patients with FGF-23 level above median regardless of phosphate levels ($p = 0.03$).
Conclusions In HD patients, plasma FGF-23 level is superior to phosphate in the prediction of coronary artery calcification. However, FGF-23 is not associated with carotid artery atherosclerosis in HD patients.

Keywords Fibroblast growth factor-23 · Coronary artery calcification · Carotid artery intima-media thickness · Hemodialysis

Introduction

Cardiovascular (CV) disease is the major cause of mortality in chronic kidney disease (CKD) patients. The CV mortality rate, even adjusted with traditional risk factors such as age, gender and diabetes, is still tenfold to 20-fold higher in dialysis patients compared to the general population [1–3]. Atherosclerosis is the main pathophysiological mechanism associated with the development and progression of CV disease in patients with CKD [4, 5]. Also, in uremic patients, vascular smooth muscle cells are transformed into osteoblast-like cells leading to vascular calcification [6–8]. This is especially important when we consider that vascular calcification is a prominent risk factor for cardiovascular mortality in CKD patients [9, 10].

Fibroblast growth factor-23 (FGF-23) is a protein mainly synthesized and secreted by osteoblasts to maintain normal

✉ Mehmet Nuri Turan
mnturan@mail.com

1 Division of Nephrology, Ege University School of Medicine, 35100 Bornova, Izmir, Turkey

2 Department of Biochemistry, Dokuz Eylul University School of Medicine, Izmir, Turkey

3 Department of Radiology, Ege University School of Medicine, Izmir, Turkey

4 Division of Nephrology and Clinical Immunology, RWTH University of Aachen, Aachen, Germany

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levels of phosphate by providing renal excretion of phosphate and inhibiting calcitriol synthesis [11–13].

It has been previously reported that increased FGF-23 levels are independently associated with mortality and cardiovascular (CV) outcomes in patients with CKD [14–20]. However, there are limited and conflicting data on the association between FGF-23 and carotid artery atherosclerosis in patients with CKD [21–26]. Most of the reports indicate an association between FGF-23 and the presence or severity of coronary artery calcification (CAC); however, there are also some negative studies [16, 27–34]. In this study, the relationship between plasma FGF-23 levels and coronary artery calcification (CAC) and carotid artery intima-media thickness (CA-IMT) in hemodialysis (HD) patients was investigated.

Methods

Patients and study design

The study group consisted of a subgroup of patients participated in a clinical trial, EGE study [35]. The EGE study was a randomized controlled trial to explore the effects of membrane flux and dialysate purity on cardiovascular outcomes. Main inclusion criteria were age 18–80 years and thrice-weekly HD; main exclusion criterion was life expectancy less than a year. Multi-slice computerized tomography and carotid artery ultrasonography were carried out in study participants according to the study protocol.

In this cross-sectional study, plasma intact FGF-23 levels were measured in randomly selected 229 patients who underwent both examinations within the period of 3 months and who had plasma samples stored at −80 °C.

Demographical, clinical and laboratory data were collected from patients' charts. The local ethics committee approved the study, and informed consent was obtained from all patients. The study was performed according to the recommendations of the Declaration of Helsinki.

Laboratory measurements

Blood samples were collected at the beginning of the HD session under fasting conditions. Until use, all samples were kept at −80 °C. All biochemical parameters were determined by standard auto-analyzers (Architect C8000 and CELL-DYN 3700) in the same central laboratory registered in external quality-control programs.

Plasma concentrations of intact FGF-23 were measured in duplicate using an ELISA kit according to the manufacturer's protocol (Immutopics, San Clemente, CA). Intra- and interassay coefficients of variation (CV) were below 5 and 7 %.

Measurement of coronary artery calcification

Multi-slice computerized tomography scans were performed with a 16-slice technique (Aquilion 16, Toshiba Medical Systems, Tokyo, Japan). All scans with slices of 3.0 mm thickness were acquired under the following condition: 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 electrocardiography (ECG) monitoring. Calcium scoring was performed on the reconstructed image sets with commercially available software (Terarecon 3.4.2.11, CA, USA). Threshold calcium determination was set using a density of at least 130 Hounsfield units. CAC score (CACs) was calculated by summing the calcification score in the left main, the left anterior descending, the left circumflex and the right coronary artery. CACs was blindly evaluated by the same radiologist, according to the method described by Agatston et al. [36].

Carotid artery intima-media thickness measurement

Ultrasonographic studies on common carotid arteries were carried out by grayscale high-resolution color Doppler ultrasound (ATL HDI 5000 scanner Philips, ATL ultrasound, Bothell, WA, ABD) equipped with 5- to 12-MHz linear transducer. The same operator performed all procedures on both sides of two longitudinal images of the each common carotid artery on the morning. Average of two CA-IMT values from each side was used to calculate mean CA-IMT. Intraobserver coefficient of variation was 2.8 %.

Statistical analysis

The Kolmogorov–Smirnov test was used to determine the normality of the distribution of variables. The parameters that were not distributed normally are given as median value with interquartile ranges and compared with nonparametric tests. Other parameters are expressed as mean ± SD. p value <0.05 was considered as statistically significant. Spearman's analysis was used to assess correlations of FGF-23 and other variables. Differences between more than two groups were analyzed by ANOVA. Multivariate ordinal regression analysis was used to determine the predictors for the severity of CACs (grouped as CAC = 0, 1–100, 101–400 and >400). All statistical analyses were performed using SPSS, version 15.0 (Chicago, IL, USA).

Results

The etiology of end-stage renal disease was diabetic nephropathy in 24 % of the study patients, hypertensive

renal disease in 15 %, polycystic kidney disease in 6 %, chronic glomerulonephritis in 6 %, other causes in 12 % and unknown in 37 %. Sixty-eight percent of the patients were taking phosphate binders (all calcium based); the use of vitamin D was 8.3 %. All of the patients were on thrice-weekly 4-h HD treatment with standard bicarbonate dialysis (Na 138 mmol/l, K 2.0 mmol/l, Ca 1.50–1.75 mmol/l, Mg 0.5 mmol/l, Cl 109 mmol/l, HCO_3 32 mmol/l, acetate 3 mmol/l, glucose 5.5 mmol/l). Seventy-five percent of the patients were dialyzed with a high-flux membrane and 25 % with a low-flux membrane (FX 60/80 and F7/F8 HPS, respectively, Fresenius Medical Care, Bad Homburg, Germany). There was no difference between CAC scores and also FGF-23 subgroups regarding the frequency of high-flux dialysis.

The clinical characteristics and laboratory data of the study population ($n = 229$) are summarized in Table 1. Briefly, mean age was 58.7 ± 14.2 years and prevalence of diabetes 24 %. Prevalences of patients with serum phosphate levels above 5.5 mg/dl and a calcium-phosphate product above >55 mg^2/dl^2 were 24 and 18 %, respectively.

Mean FGF-23 level was 251 ± 385 pg/ml (14.8–1297.1); median FGF-23 was 53.5 pg/ml (IQR 30.8–249.5). Mean CACs was 478 ± 984. The frequency of patients with severe calcification (CACs > 400) was 28.8 %; 27.5 % of cases had no calcification. Mean CA-IMT was 0.78 ± 0.20 mm, and the presence of carotid plaques was 51 % with a mean length 2.1 mm (0.60–4.40 mm).

Table 2 shows the clinical and laboratory characteristics of the patients according to the FGF-23 tertiles. The patients with higher FGF-23 levels had higher CACs, phosphate, calcium-phosphate product, serum 25-OH vitamin D, albumin and creatinine levels. There was no difference among the groups with regard to serum PTH, the use of vitamin D and calcium-based phosphate binders. Mean CA-IMT values and the frequency of carotid artery plaques were not different among the tertiles.

In univariate analysis, plasma FGF-23 level was positively correlated with calcium-phosphate product ($r = 0.465$, $p < 0.001$), serum phosphate ($r = 0.397$, $p < 0.001$), calcium ($r = 0.337$, $p < 0.001$), 25-OH vitamin D level ($r = 0.245$, $p < 0.01$) and CACs ($r = 0.218$, $p = 0.001$). While there was a negative correlation between FGF-23 and serum alkaline phosphatase levels (-0.192, $p = 0.006$), no correlation was found between serum PTH level and FGF-23. Neither CA-IMT nor the presence of carotid artery plaques correlated with FGF-23 levels.

In a linear regression analysis using logFGF-23 as dependent variable, serum phosphate ($t = 6.39$, $p < 0.001$), calcium ($t = 4.47$, $p < 0.001$), 25-OH vitamin D level ($t = 3.60$, $p < 0.001$) and CACs ($t = 3.39$, $p = 0.001$) were identified as independent predictors for FGF-23 level (model $r^2 = 0.39$, $p = 0.001$).

Demographic and laboratory parameters of the study population stratified according to CACs quartiles are summarized in Table 1. The patients with higher CACs were older, more likely to be male, had higher frequency of diabetes and CV disease history, serum calcium, calcium-phosphate product and higher FGF-23 levels, compared to the patients with lower CACs.

In an adjusted ordinal regression analysis, FGF-23 level was an independent predictor for severe CACs together with age, gender, presence of diabetes, time on dialysis and CA-IMT (model $r^2 = 0.44$, $p < 0.001$) (Table 3). Every 50 pg/ml increase in FGF-23 level was associated with an 17 % increase in risk for severe CAC (RR 1.17, 95 % CI 1.05–1.30, $p = 0.003$).

When patients were categorized according to the median levels of serum phosphate (4.9 mg/dl) and FGF-23 (53.5 pg/ml), the mean CACs was markedly higher in patients with high FGF-23 irrespective of phosphate levels ($p = 0.03$) (Fig. 1).

Discussion

In this cross-sectional study, our results show that there was no association between plasma FGF-23 level and CA-IMT in HD patients. On the other hand, increased FGF-23 levels were associated with the severity of CACs together with traditional risk factors. The association between FGF-23 and CAC was found to be independent of serum phosphate levels.

It is well known that phosphate may directly induce vascular smooth muscle cells to transform into osteoblast-like cells, which contribute to vascular calcification in CKD [6–8]. In our study, there was no significant difference in serum phosphate levels between the different CACs groups. Our study population, however, is notable for relatively stringent phosphate control. As spot phosphate levels may fluctuate by several factors such as current diet, adequacy of dialysis and treatment, we speculate that increased FGF-23 levels are a consequence of chronic phosphate accumulation in body. Another explanation of our finding is that elevated FGF-23 might be directly responsible for vascular calcification. Initially, no calcification was identified with FGF-23 overexpression in nonuremic transgenic animals [37–39]. However, the investigation of direct effects of FGF-23 on vascular calcification in the aorta of both normal and uremic rats showed that FGF-23 increased phosphate-induced vascular calcification by promoting osteoblastic differentiation involving the ERK1/2 pathway, while FGF-23 alone without the high phosphate concentration of the medium had no effect on vascular calcification [40]. In contrast, exogenous FGF-23 did not induce calcification of cultured human vascular smooth muscle cells regardless of

Table 1 Demographic and biochemical characteristics of the study population stratified according to CAC score group

	All patients	Coronary artery calcification score					Group difference
		Group 1 0 $n = 63$	Group 2 1–100 $n = 53$	Group 3 101–400 $n = 47$	Group 4 >400 $n = 66$	p for trend	
Age (years, median—IQR)	60.8 (50.7–69.4)	48.7 (38.5–58.5)	62.6 (55.8–68.9)	60.5 (54.2–68.4)	67.8 (56.8–73.8)	<0.001	1 versus other groups
Gender (male, %)	51	41	38	59	56	0.003	1 versus 3, $p = 0.01$; 2 versus 3, $p = 0.006$
Diabetes (%)	24	3	25	38	32	<0.001	1 versus 2, 3, 4, $p < 0.001$
CVD history (%)	20	5	23	22	32	0.001	1 versus 2, 3, 4, $p = 0.001$
Hypertension (%)	18.1	12.7	15.3	21.7	23.0	0.39	–
Smoking (%)	31	19	29	37	39	0.06	–
Time on dialysis (months, median—IQR)	41.3 (21.5–68.1)	34.9 (21.4–62.3)	31.6 (21.8–57.6)	52.1 (24.0–85.2)	51.2 (17.9–77.6)	0.07	–
Body mass index (kg/m^2, mean ± SD)	24.3 ± 4.3	23.4 ± 4.0	25.2 ± 4.8	24.0 ± 3.9	24.7 ± 4.2	0.15	–
Equilibrated Kt/V (median—IQR)	1.49 (1.04–2.49)	1.51 (1.37–1.70)	1.53 (1.36–1.73)	1.42 (1.30–1.55)	1.47 (1.32–1.63)	0.10	–
Creatinine (mg/dl, mean ± SD)	8.4 ± 1.7	8.7 ± 1.9	8.4 ± 1.8	8.6 ± 1.5	8.0 ± 1.5	0.11	–
Hemoglobin (g/dl, mean ± SD)	11.0 ± 1.2	10.8 ± 1.3	10.9 ± 1.0	11.3 ± 1.3	10.9 ± 1.3	0.38	–
C-reactive protein (mg/dl, median—IQR)	0.67 (0.33–1.42)	0.47 (0.21–1.05)	0.70 (0.34–1.28)	0.76 (0.32–1.48)	0.79 (0.43–1.63)	0.28	–
Albumin (g/dl, mean ± SD)	3.92 ± 0.24	3.9 ± 0.2	3.9 ± 0.2	3.9 ± 0.2	3.8 ± 0.2	0.42	–
Calcium (mg/dl, median—IQR)	9.09 (8.70–9.57)	8.94 (8.68–9.37)	8.95 (8.67–9.28)	9.11 (8.67–9.55)	9.48 (8.85–10.09)	0.01	4 versus others, $p = 0.01$
Phosphate (mg/dl, mean ± SD)	4.89 ± 1.02	4.9 ± 0.9	4.6 ± 1.2	4.9 ± 0.9	5.0 ± 0.9	0.16	–
Calcium-phosphate product (mg^2/dl^2, median—IQR)	44.4 (38.3–51.2)	44.2 (38.5–51.3)	41.2 (32.9–46.2)	44.2 (38.6–50.4)	48.4 (41.1–55.4)	0.04	4 versus 2, $p = 0.02$
25(OH) vitamin D (ng/mL, median—IQR)	33.4 (22.0–51.8)	33.1 (22.5–56.9)	32.0 (18.1–42.2)	46.9 (22.8–63.9)	32.8 (21.9–49.7)	0.14	–
Alkaline phosphatase (U/L, median—IQR)	98 (74–130)	97 (69–133)	106 (87–134)	107 (69–142)	94 (70–126)	0.31	–
Parathyroid hormone (pg/ml, median—IQR)	151 (59–257)	156 (59–261)	118 (63–236)	211 (97–303)	132 (44–277)	0.17	–
Total cholesterol (mg/dl, mean ± SD)	172 ± 42	167 ± 34	173 ± 42	174 ± 52	173 ± 42	0.78	–

Table 1 continued

	All patients	Coronary artery calcification score					Group difference
		Group 1 0 $n = 63$	Group 2 1–100 $n = 53$	Group 3 101–400 $n = 47$	Group 4 >400 $n = 66$	p for trend	
Triglyceride (mg/dl, median—IQR)	159 (111–206)	152 (96–206)	160 (109–211)	170 (116–219)	156 (110–194)	0.71	–
Ca-based phosphate binder dose (g/day, median—IQR)	3.0 (0.0–6.0)	3.0 (0.0–6.0)	3.0 (0.0–6.0)	3.0 (2.0–6.0)	3.0 (0.0–6.0)	0.50	–
Calcitriol use (%)	8.3	6.3	7.5	4.2	13.6	0.26	–
CA-IMT (mm, median—IQR)	0.75 (0.65–0.92)	0.65 (0.55–0.72)	0.76 (0.67–0.91)	0.72 (0.65–0.91)	0.87 (0.76–1.00)	<0.001	1 versus 2, 3, 4, $p < 0.001$; 3 versus 4, $p = 0.008$
Carotid artery plaques (%)	51	20	46	60	79	<0.001	1 versus 2, 3, 4, $p < 0.001$; 2 versus 4, $p = 0.002$
FGF-23 (pg/ml, median—IQR)	53.5 (30.8–249.5)	38.9 (25.0–139.2)	49.5 (33.3–118.8)	46.9 (31.9–301.6)	117 (34.7–961.1)	0.01	4 versus 1, $p = 0.03$
CACs (median—IQR)	98 (0–531)	0	25 (7–51)	193 (145–263)	954 (629–1814)	–	

CVD cardiovascular disease, *CA-IMT* carotid artery intima-media thickness, *FGF-23* fibroblast growth factor 23, *CACs* coronary artery calcification score

the phosphate concentration of the medium [34]. Similarly, no effect of FGF23 on vascular calcification was shown in bovine vascular smooth muscle cells [41]. Thus, current experimental data on possible direct effects of FGF-23 on calcification in uremia are inconsistent. Interestingly, calcification increased with a monoclonal anti-FGF-23 antibody used to reduce high serum FGF-23 levels in CKD rats [42]. However, in that study, enhanced calcification may have been caused by the phosphate retention as a result of FGF-23 blockade. In any case, our results indeed suggest that FGF-23 outperforms spot phosphate levels in terms of predicting calcification in HD patients.

Vascular calcification is associated with some risk factors such as age, male gender and diabetes in the normal population [43]. However, a number of additional risk factors have been described in HD patients, who have more common and severe vascular calcification compared to the general population [44]. FGF-23 was an independent predictor for severe CACs together with age, gender, presence of diabetes, time on dialysis and CA-IMT in our study. While atherosclerosis and inflammation are associated with intimal calcification, abnormal changes in mineral metabolism are considered to be the most important factor for medial calcification [45, 46]. FGF-23 is associated with abnormalities in mineral metabolism occurring in CKD and increases progressively in the process of CKD [47, 48]. We also found serum phosphate, calcium, 25-OH vitamin D levels and CACs as independent predictors for logFGF-23 levels.

Increased FGF-23 is now widely accepted as an independent predictor of mortality and cardiovascular outcomes [14–20] and progression of kidney disease [48]. With regard to vascular calcification, Jean et al. [49] showed an association of FGF-23 with peripheral calcification and Nasrallah et al. with aortic calcification [50]. However, the studies on association between FGF-23 and the presence or severity of CAC in CKD have been conflicting [16, 27–34].

In patients with normal renal function, no correlation was found between FGF-23 and CAC [30]. Gutierrez et al. [16] showed that highest tertile of FGF-23 was associated with 2.4-fold increased risk CAC $\geq$ 100 versus <100 U compared with the lowest tertile in patient with CKD but not on dialysis. However, the association was lost after adjustment for other variables. In an unselected population whose glomerular filtration rate was between 30 and 90 ml/min/1.73 m^2, FGF-23 was identified as an independent predictor of coronary artery disease score assessed by coronary angiography [27]. In two prospective studies, FGF-23 was found to be associated with CAC progression in dialysis patients without any relationship between baseline FGF-23 and CAC [31, 32]. In another study evaluating the progression of CAC in 47 prevalent HD patients, those with CACs > 30 U compared with CACs < 30 U had significantly higher FGF-23 levels, but no multivariate

Table 2 Demographic and biochemical characteristics of the study population stratified according to FGF-23 tertiles

	FGF-23 tertiles				Group difference
	Group 1 <35.5 pg/ml $n = 76$	Group 2 35.5–123.4 pg/ml $n = 77$	Group 3 >123.4 pg/ml $n = 76$	p value	
Age (years, median—IQR)	60.9 (45.4–70.0)	61.5 (49.9–70.0)	59.8 (51.7–68.9)	0.87	–
Gender (male, %)	56.6	46.5	50.0	0.38	–
Diabetes (%)	30.3	22.1	18.4	0.21	–
CVD history (%)	17.1	17.3	26.3	0.27	–
Hypertension (%)	16.6	11.8	25.6	0.07	–
Smoking (%)	20.8	31.1	39.7	0.04	1 versus 3, $p = 0.03$
Time on dialysis (months, median—IQR)	38.8 (21.5–63.4)	42.3 (23.9–68.3)	38.1 (17.0–72.0)	0.84	–
Body mass index (kg/m^2, mean ± SD)	23.9 ± 4.1	24.4 ± 4.6	24.7 ± 4.0	0.47	–
Equilibrated Kt/V (median—IQR)	1.52 (1.37–1.71)	1.49 (1.35–1.67)	1.44 (1.30–1.63)	0.07	–
Creatinine (mg/dl, mean ± SD)	7.89 ± 1.42	8.63 ± 1.82	8.82 ± 1.90	0.01	1 versus 2 and 3 ($p = 0.03$)
Hemoglobin (g/dl, mean ± SD)	11.4 ± 1.4	11.3 ± 1.4	11.5 ± 1.6	0.31	–
C-reactive protein (mg/dl, median—IQR)	0.64 (0.33–1.18)	0.76 (0.33–1.52)	0.66 (0.31–1.51)	0.31	–
Albumin (g/dl, mean ± SD)	3.88 ± 0.26	3.93 ± 0.22	3.93 ± 0.24	0.01	1 versus 2 and 3, $p = 0.01$
Calcium (mg/dl, median—IQR)	8.83 (8.50–9.24)	9.10 (8.76–9.45)	9.42 (8.94–9.97)	0.001	<0.001 for trend
Phosphate (mg/dl, mean ± SD)	4.46 ± 0.86	4.80 ± 0.88	5.41 ± 1.08	<0.001	<0.001 for trend
Calcium-phosphate product (mg^2/dl^2, median—IQR)	41.3 (33.5–44.6)	43.6 (38.6–49.1)	51.2 (43.5–59.9)	<0.001	<0.001 for trend
25(OH) vitamin D (ng/ml, median—IQR)	32.5 (18.2–50.7)	30.9 (22.0–40.1)	46.2 (29.1–65.0)	0.03	3 versus 1 and 2, $p = 0.01$
Alkaline phosphatase (U/L)	114 (81–147)	100 (71–130)	91 (64–122)	0.06	–
Parathyroid hormone (pg/ml, median—IQR)	158 (73–259)	147 (61.5–266)	145 (53.7–300)	0.96	–
Total cholesterol (mg/dl, mean ± SD)	183 ± 46	186 ± 47	190 ± 40	0.56	–
Triglyceride (mg/dl, median—IQR)	132 (86–188)	171 (114–213)	159 (119–210)	0.07	–
Ca-based phosphate binder dose (g/day, median—IQR)	3.0 (0.0–6.0)	3.0 (0.0–6.0)	3.0 (0.0–6.0)	0.79	–
Calcitriol use (%)	5.5	3.8	15.3	0.01	3 versus 2, $p = 0.04$
CA-IMT (mm, median—IQR)	0.77 (0.65–0.92)	0.72 (0.65–0.89)	0.75 (0.65–0.93)	0.44	–
Carotid artery plaques (%)	46	51	57	0.38	–
CAC scores (median—IQR)	47.5 (0.0–371.0)	55.9 (4.0–322.8)	189 (0–832)	0.03	3 versus 1 and 2, $p = 0.01$
FGF-23 (pg/ml, median—IQR)	25.4 (22.8–29.9)	50.8 (39.3–75.1)	525 (210–1137)	–	

CVD cardiovascular disease, *CA-IMT* carotid artery intima-media thickness, *FGF-23* fibroblast growth factor 23, *CACs* coronary artery calcification score

Table 3 Predictors for the severity of CAC score in study population

Independent variables	RR	95 % CI	p value
Age (per 1 year)	1.07	1.04–1.11	<0.001
Diabetes (vs. nondiabetes)	2.17	1.09–4.28	0.0
Time on dialysis (month)	1.01	1.00–1.02	<0.001
CA-IMT thickness (per 0.1 mm)	8.58	1.32–56.2	0.001
Male (vs. female)	4.14	2.01–8.51	<0.001
FGF-23 (per 50 pg/ml)	1.17	1.05–1.30	0.003

Included variables into model: age, gender, diabetes, cardiovascular disease history, smoking, time on dialysis, calcium, phosphate, calcium-phosphate product, 25-OH vitamin D level, CA-IMT, CA plaques and FGF-23 (model r^2:0.44, $p < 0.001$)

RR relative risk, *CI* confidence interval, *CV* cardiovascular, *CA-IMT* carotid artery intima-media thickness

analysis was provided [28]. In a small group of pediatric HD patients, FGF-23 together with phosphate was the most significant predictor of CAC after multivariate analysis [29]. In patients with GFR higher than 60 mL/min/1.73 m^2, serum FGF-23 levels were independently associated with coronary calcification [33]. In 1501 patients from the Chronic Renal Insufficiency Cohort (CRIC) study, the prevalence of CAC (scores of >0) was significantly higher in the higher quartiles of FGF23, but the association did not persist after adjustment for traditional CV risk factors [34]. Given that mineral metabolism disturbances, vascular calcification and increases in FGF-23 all are more prominent in the dialysis population [14, 16, 51–54], the relationship between calcification and FGF-23 could be more detectable than other CKD stages.

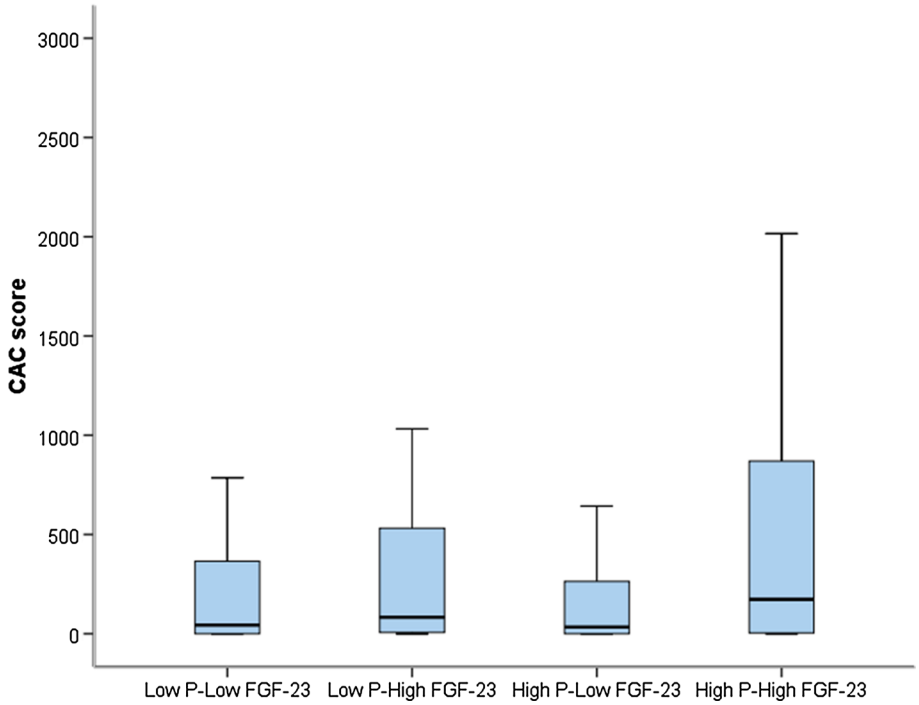

Fig. 1 Coronary artery calcification scores in phosphate and FGF-23 combined subgroups ("low," below median value; "high," equal or above median value; median levels of phosphate and FGF-23 4.90 mg/dl and 53.5 pg/ml, respectively)

There are limited data on the association between FGF-23 level and carotid artery atherosclerosis in patients with CKD [21–26]. CA-IMT was found to be positively associated with plasma FGF-23 levels in 62 peritoneal dialysis patients [21]. A study in 129 maintenance HD patients showed that FGF-23 levels were independently associated with CA-IMT [22]. However, FGF-23 was found to be inversely correlated with CA-IMT and a negative predictor of an increase in CA-IMT in 196 maintenance HD patients [23]. In 67 patients on peritoneal dialysis, FGF-23 was not associated with CA-IMT [24]. Also, no association between CA-IMT and FGF-23 was detected in 60 patients on HD [25]. Similarly, we found that plasma FGF-23 level was not associated with CA-IMT. In a subgroup of the Multi-Ethnic Study of Atherosclerosis (MESA) population [26], a prospective cohort study of cardiovascular disease among 6814 community-living individuals, the association of FGF-23 with carotid IMT was not statistically significant. At the same time, higher FGF-23 concentrations were found to be associated with significantly greater CACs in unadjusted and adjusted analysis, which confirms our results.

Our study has several limitations. First, the study was cross-sectional. CACs, CA-IMT and FGF-23 levels can change over time. No causal link could be provided. Additionally, our study was performed in maintenance dialysis patients. Therefore, there may be a selection bias of including prevalent HD patients (survivors).

In conclusion, our study shows a relationship between plasma FGF-23 levels and CACs, independently of phosphate levels. Further large-scale studies exploring the role of FGF-23 in calcification in dialysis population and whether FGF-23 is a potential pathophysiological mechanism or a marker of calcification may help us to clarify this issue.

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Compliance with ethical standards

Conflict of interest EO is a member of scientific board of Fresenius Medical Care, Turkey. The other authors declare no conflict of interest.

Ethics committee approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from patients who participated in this study.

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