FGF-23 and the Progression of Coronary Arterial Calcification in Patients New to Dialysis

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Summary

Background and objective Fibroblast growth factor 23 (FGF-23), a regulator of phosphorus metabolism, is a risk marker in CKD. FGF-23 has been associated with coronary arterial calcification (CAC), but it is not known whether FGF-23 predicts CAC progression in CKD. The aim of this study was to evaluate the association of FGF-23 with CAC progression in advanced CKD.

Design, setting, participants, & measurements FGF-23 levels and CAC were measured by electrocardiography-triggered multislice computed tomography in 99 individuals initiating dialysis. Patients were enrolled in the study from April 2008 to July 2010. CAC was calculated using Agatston and calcium volume score. Sixty-seven study participants had repeat CAC measures at 1 year. Linear regression was used to assess the association of FGF-23 with CAC.

Results The mean age of study participants was 50 years; 33% were women, and 64% were black. The median FGF-23 level was 1238 relative units (RU)/ml (interquartile range, 515–2218 RU/ml). According to Agatston score, FGF-23 was not associated with baseline CAC (P=0.14) but was significantly associated with CAC progression. There was a 192.3–Agatston unit change in CAC score per 1-SD change in FGF-23 (P=0.008) in models adjusting for known risk factors for CAC and serum phosphate. This association persisted after adjustment for high-sensitivity C-reactive protein, 25-OH vitamin D levels, and the use of phosphorus binders. Results were similar when change in calcium volume score was used.

Conclusions In individuals with advanced CKD, serum FGF-23 is strongly associated with CAC progression. FGF-23 may be a marker of cardiovascular risk in CKD.

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Introduction

Individuals with CKD have a more than eight-fold higher risk for cardiovascular mortality than those with normal kidney function, and CKD is an important contributor to the global burden of cardiovascular disease (1). Traditional risk factors, such as long-standing hypertension and diabetes mellitus, do not adequately predict cardiovascular events and mortality in this population. Accelerated arterial calcification, which has been related to alterations in kidney function and mineral metabolism, is one mechanism by which individuals with CKD are predisposed to cardiovascular events (2,3).

Hyperphosphatemia is associated with cardiovascular events in both CKD and non-CKD populations (4,5). Fibroblast growth factor 23 (FGF-23) is a circulating hormone that stimulates urinary phosphate excretion and decreases dietary phosphorus absorption (6), thereby serving to normalize serum phosphate concentration. FGF-23 levels are higher in individuals undergoing dialysis and are strongly associated with mortality, independent of phosphate concentration (7,8). FGF-23 has been postulated to play a role in the development of coronary arterial calcification (CAC) and atherosclerosis, which may underlie its prognostic significance (9–11). However, available cross-sectional data are conflicting and prospective data are lacking (12).

CAC progression is a quantifiable marker of atherosclerosis, and its progression has been associated with mortality in ESRD (13). Given the lifelong progression of atherosclerotic disease, cross-sectional studies assessing the relationship between FGF-23 and atherosclerosis may be confounded by exposure history that cannot be accounted for with single measurements (14). Therefore, prospective studies assessing the progression of atherosclerosis as a function of FGF-23 levels are needed. In this study, we tested the hypothesis that elevated FGF-23 levels are associated with CAC progression in a cohort of individuals who are new to dialysis.

Materials and Methods

We investigated 99 adults new to dialysis who agreed to participate in a prospective study to identify

risk factors for CAC progression and had FGF-23 measured at their baseline visit. Patient enrollment began in April 2008 and ended in July 2010. A total of 358 individuals were approached about the study, of whom 251 declined participation. The most commonly cited reasons for nonparticipation were feeling overwhelmed with starting dialysis, not wanting to add hospital visits, and lack of transportation or lack or interest in research. A total of 107 individuals provided consent, but 5 of those individuals did not complete the first study visit.

Participants were excluded if they had a history of coronary revascularization or weighed more than 350 pounds (for technical reasons). The first research visit occurred within 4 months of dialysis initiation, and a follow-up visit occurred at least 12 months later. The results of FGF-23 testing were not provided to the clinicians taking care of study participants. However, clinicians were informed about CAC measurements >400 Agatston units (AU) because this value has been associated with increased risk for death and the institutional review board required this notification. All participants provided written informed consent prior to enrollment in the study, and the institutional review board of the University of Pennsylvania approved the protocol.

Body height, weight, and BP measurements were obtained. BP was measured three times in the seated position, and the mean of the three values was calculated. Body mass index was calculated as weight in kg divided by height squared (m^2). Comorbid conditions were assessed by self-report, and medications were reviewed. Cardiovascular disease was defined as history of coronary artery disease, cerebrovascular disease, or peripheral vascular disease.

CAC was measured by electrocardiography-triggered multislice computed tomography (CT). All CT scans were obtained on the same dual-source 64-slice scanner (Somatom Definition, Siemens Medical Solutions, Malvern, PA) using contiguous 3-mm-thick transverse images. CT scans were assessed for quality and were evaluated quantitatively by a single radiologist (H.L.) who was blinded to the other study data. A total coronary calcium score was calculated for each scan by multiplying the area of the focus by a coefficient based on the density of the focus as originally described by Agatston *et al*. (15). CAC progression was evaluated as a continuous variable by calculating the difference in Agatston score between the two CT scans divided by the time between imaging. In addition, we used the square root transformed difference (SQRT) method, in which CAC progression is measured as the difference in the square root–transformed CAC score between scans. CAC progression was defined as a difference of >2.5 mm^3 (16). The SQRT method has been shown to minimize interscan variability (16).

All study samples were processed within 1 hour of collection. FGF-23 was measured in duplicate after a single thaw of stored baseline plasma specimens using a second-generation C-terminal ELISA (Immutopics). The total inter- and intra-assay coefficient of variability was 7.6% at 308 relative units (RU)/ml. The assay detects both intact FGF-23 and its C-terminal fragments. However, most circulating FGF-23 is intact, and thus the C-terminal assay measures biologically active FGF-23 (17).

For descriptive analyses, we stratified the population according to tertile of FGF-23 levels. Continuous variables are expressed as mean and SD or median (interquartile range) as appropriate. Categorical variables were expressed as counts and percentages. We tested the differences in continuous variables between FGF-23 tertiles using ANOVA and in categorical variables using the chi-squared test.

We used multivariate linear regression to assess whether FGF-23 at baseline is an independent predictor of baseline CAC score and annual CAC progression, defined by both the change in Agatston score and the SQRT method. Baseline CAC score was log transformed because of its non-normal distribution. FGF-23 was also natural log transformed to achieve normality and then standardized. The following covariates were included in the multivariable model: age, sex, race, diabetes status, smoking status, and serum phosphate. The resulting β coefficient represents the change in outcome variable per 1-unit change in the independent variable (1SD log FGF-23). Baseline CAC score was added to models examining CAC progression. High-sensitivity C-reactive protein (hsCRP), 25-OH vitamin D, and the use of phosphorus binders were added to the models in secondary analyses. We also evaluated association of FGF-23 between progressors (defined change in CAC >25%) and nonprogressors using logistic regression. Additionally, we evaluated the association of FGF-23 with incident CAC using logistic regression. Stata software, version 10.1 (Stata Corporation, College Station, TX), was used for all statistical analyses.

Results

Baseline characteristics of the entire study sample and by tertile of serum FGF-23 are shown in Table 1. The mean age of the sample was 50.1±12.7 years. Thirty-three percent of patients were women and 64% were black. Participants were dialyzed in the following manner: in-center hemodialysis (n=87), home hemodialysis (n=3), and peritoneal dialysis (n=9). The median FGF-23 level was 1238 RU/ml (interquartile range, 515–2218 RU/ml). At the baseline examination, 36% of participants had no CAC and 43% of participants had minimal CAC as defined by a CAC score < 10 AU.

Higher baseline FGF-23 levels were associated with higher serum phosphate (P=0.001) concentrations. There was no association of baseline FGF-23 and other known risk factors for coronary artery disease, including age, sex, race, BP, serum cholesterol, diabetes status, smoking status, body mass index, or hsCRP levels. There was also no association of FGF-23 level and baseline CAC score as calculated by Agatston score (P=0.14) or calcium volume score (P=0.32). Diabetes and smoking status were positively associated with baseline CAC (P<0.001 for both) in univariate models, whereas race and serum phosphate were not (P=0.14 and P=0.44, respectively). Both *a priori* specified variables and variables that were associated with baseline CAC at the <0.2 level were selected for inclusion in the multivariable model.

There was no statistically significant difference in the use of calcium-containing phosphate binders (P=0.28), activated vitamin D (P=0.79), or sevelamer use (P=0.63) by

Table 1. Demographic and physiologic characteristics for all participants and by tertiles of serum FGF-23

Characteristic	All Patients (n=99)	FGF-23			P Value
		Low (n=33)	Intermediate (n=33)	High (n=33)	
Demographic					
Age (yr)	50.0±12.8	54.0±11.2	48.4±12.4	47.7±13.6	0.09
Women (%)	32	30	40	27	0.55
Black (%)	64	64	64	64	1.00
Baseline					
Diabetes (%)	52	60	48	47	0.48
Cardiovascular disease (%)	28	24	30	30	0.82
Ever smoker (%)	52	50	47	59	0.58
Body mass index (kg/m^2)	29±6	29±5	30±5	30±7	0.62
Systolic BP (mmHg)	137±20	132±17	140±25	137±18	0.30
Diastolic BP (mmHg)	78±12	76±10	78±13	80±13	0.43
Medication use					
Statins (%)	40	45	48	27	0.17
Antihypertensive medications					
β-blockers	62	58	76	55	0.16
Diuretics	38	30	48	36	0.30
ACE inhibitors or ARBs	45	42	36	58	0.20
Phosphate binders (%)					
Calcium-containing	47	53	38	50	0.42
Non–calcium-containing	22	13	34	22	0.11
Activated vitamin D (%)	22	31	22	13	0.19
Warfarin (n)	3	0	0	3	0.05
Laboratory assessment					
Total cholesterol (mg/dl)	169±38	168±34	167±33	172±47	0.87
Parathyroid hormone (pg/ml)	253±209	213±116	296±243	253±241	0.28
Phosphate (mg/dl)	4.6±1.3	3.9±1.0	4.6±1.2	5.3±1.4	<0.001
Calcium (mg/dl)	8.8±1.1	8.7±0.9	8.7±1.0	9.0±1.2	0.53
25-OH vitamin D (ng/ml)	26.1±15.1	27.3±14.5	23.9±15.5	26.9±15.7	0.62
Albumin (g/dl)	3.5±0.6	3.5±0.6	3.5±0.5	3.5±0.6	0.90
Median hsCRP (mg/L) (IQR)	6.4 (2.6–13.9)	7.0 (3.0–13.5)	8.8 (1.5–15.1)	4.8 (2.7–10.5)	0.61
Median FGF-23 (RU/mL) (IQR)	1238 (515–2218)	432 (383–515)	1238 (958–1459)	3659 (2218–5238)	<0.001
Baseline coronary calcium assessment					
Median baseline Agatston score (Agatston units) (IQR)	26.0 (0–274.6)	26.0 (0–158.8)	16.0 (0–368.0)	58.5 (0–310.1)	0.81
Median baseline calcium volume score (IQR)	28.4 (0–210.7)	25.2 (0–143.4)	15.9 (0–270.0)	48.1 (0–256.6)	0.81

Clinical characteristics are expressed as mean ± SD, percentage, or median (interquartile range), where appropriate. ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range.

baseline CAC score. There was also no statistically significant difference in the use of calcium-containing phosphate binders (P=0.08), activated vitamin D (P=0.55), or sevelamer use (P=0.23) between those who did and did not have CAC progression. Warfarin use was not associated with baseline CAC (P=0.76). No individuals taking warfarin (n=3) had follow-up scans, and therefore they were not included in analyses of CAC progression.

Repeated measures of CAC were available in 67 individuals. The remaining participants (n=32) did not undergo follow-up CAC assessment for the following reasons: interim kidney transplantation (n=7) or coronary revascularization (n=3), claustrophobia (n=1), death (n=7), and lack of interest in attending a second research visit (n=14).

The average interscan time was 1.1±0.3 years. There was no statistically significant difference in baseline intact parathyroid hormone, calcium, phosphorus, CRP, and FGF-23 levels in those who underwent follow-up scanning and those who did not (Supplemental Table 1). However, individuals who underwent follow-up CAC testing had lower baseline CAC than those who did not (164.1 AU versus 437.6 AU; P=0.01). The median annual CAC progression was 16 AU (interquartile range, 0–105 AU). Twenty-three participants (37%) met the definition of CAC progression. More than a third of participants (10 of 27 individuals) with no evidence of CAC at the baseline scan developed CAC. However, only three had a CAC progression >3 AU (range, 0.8–375.4 AU).

In unadjusted models using the Agatston score, baseline CAC was the only variable associated with CAC progression (β=73.41; P<0.002). In multivariable models adjusting for baseline Agatston score, age, sex, race, smoking status, diabetes status, and serum phosphate, only FGF-23 remained significantly associated with CAC progression as defined by change in Agatston score (β=192.30; P=0.008) (Table 2). Therefore, there was a 192.3-AU change in CAC score per 1-SD change in FGF-23.

The association of FGF-23 with CAC progression persisted after further adjustment for the inflammatory marker hsCRP (β=174.75; P=0.02), 25-OH vitamin D levels (β=193.32; P=0.009), the use of phosphorus binders (β=193.90; P=0.007), or all of these factors together in one model (β= 177.88; P=0.02). Results were not substantively different in models that used calcium volume score instead of Agatston score (Table 2).

We found a nonsignificant association between progressors and FGF-23 (incidence rate ratio, 1.11; 95% confidence interval, 0.96–1.29; P=0.15). Of 27 participants with no calcification at baseline, 10 developed CAC at the time of the second CT examination. FGF-23 was significantly associated with development of incident CAC (incidence rate ratio, 2.26; 95% confidence interval, 1.28–3.99; P=0.005) after multivariable adjustment.

Discussion

We report that FGF-23 is an independent predictor of CAC progression in patients with advanced CKD initiating dialysis. To our knowledge, this is the first study to report this finding. This finding persisted after adjustment for conventional cardiovascular risk factors and for the inflammatory marker hsCRP. Of note, FGF-23 remained a significant predictor of CAC progression after further adjustment for serum phosphate and vitamin D levels, which have been associated with CAC in other studies (4,5,18). In contrast, we found no relationship between FGF-23 levels and prevalent CAC.

CAC predicts future cardiovascular events, including myocardial infarction, and cardiovascular mortality (19). Because atherosclerosis is a dynamic process, CAC progression may be a better marker of risk than is baseline CAC (13,20). Individuals with CKD are prone to the development of arterial calcification, which progresses rapidly in those with advanced kidney impairment, leading to an increased risk for cardiovascular morbidity and mortality (21). Therefore, CAC progression is an important marker of cardiovascular risk in this population.

FGF-23 is produced predominantly by osteocytes and osteoblasts (22) and exerts its effects *via* FGF receptors, which have been identified in multiple organ systems (23). FGF-23 also decreases kidney production of 1,25 hydroxyvitamin D, thereby decreasing the absorption of dietary phosphorus (23). FGF-23 increases early in CKD and maintains normophosphatemia by increasing urinary phosphate excretion (22–24). This mechanism is impaired as CKD progresses; therefore, hyperphosphatemia is common in severe CKD, including in patients undergoing dialysis. Hyperphosphatemia has been implicated in arterial calcification by inducing osteogenic metaplasia of vascular smooth muscle cells and is strongly associated with cardiovascular events (2,25). However, FGF-23 has multiple "off-target" or systemic effects and is probably not solely a marker of phosphate imbalance (23).

FGF-23 may be involved in the development of cardiovascular disease in individuals with CKD (7–9). Experimental data support a role for FGF-23 in the development of left ventricular hypertrophy, which is a known risk factor for mortality (26). In addition, the FGF-23 system may act on the vasculature, providing a direct link to the development of calcification. Mice deficient in klotho, a coreceptor for FGF-23, develop severe vascular calcification, while transgenic mice overexpressing klotho have less calcification than wild-type mice with CKD (27). CKD is a state of klotho deficiency, which may contribute to accelerated vascular calcification (27). Members of the FGF system

Table 2. Association of baseline factors with baseline coronary arterial calcification and progression of calcification by Agatston and calcium volume scores

Variable	Baseline CAC		CAC Progression by Agatston Score		CAC Progression by Calcium Volume Score[a]	
	Coefficient ± SE	*P* Value	Coefficient ± SE	*P* Value	Coefficient ± SE	*P* Value
Age	0.04±0.02	0.06	6.20±5.74	0.28	0.12±0.06	0.06
Female sex	−0.09±0.48	0.86	−161.76±122.10	0.19	−1.96±1.34	0.15
Black race	−1.22±0.48	0.01	164.15±131.65	0.22	2.58±1.44	0.08
Diabetes status	1.83±0.47	<0.001	76.29±129.13	0.56	1.78±1.41	0.21
Smoking status[b]	2.04±0.50	<0.001	−75.36±140.62	0.59	−2.44±1.54	0.12
Baseline CAC (log)	–	–	56.33±29.15	0.06	0.67±0.32	0.04
Serum phosphate	−0.38±0.19	0.05	−97.90±55.42	0.08	−0.62±0.61	0.31
FGF-23 (per 1-SD change)	0.38±0.25	0.14	192.30±69.97	0.008	2.04±0.77	0.01

Model for baseline coronary arterial calcification (CAC) adjusted for age, sex, race, smoking status, diabetes status, and serum phosphate. Models for CAC progression adjusted for log (baseline Agatston score), age, sex, race, smoking status, diabetes status, and serum phosphate. FGF-23, fibroblast growth factor 23.

[a]CAC progression defined as the difference in the square root-transformed CAC score between scans.

[b]Defined as current or prior smoking versus never smoking.

have also been found in pathology specimens of human aorta, although confirmatory studies are lacking (28).

Some cross-sectional studies have found an association between FGF-23 and the presence or severity of CAC (9–11,29), but this is not a universal finding (12). In a study of 47 prevalent hemodialysis patients (mean dialysis vintage, 49 months), Kurnatowska and colleagues demonstrated an association between FGF-23 and prevalent CAC in univariate analysis (11). There was no adjustment for other known risk factors. Srivaths and colleagues showed a similar association in the pediatric population (29). FGF-23 has also been associated with the severity of coronary artery disease on coronary angiography (10). In the largest study to date of FGF-23 and CAC, Gutiérrez *et al.* (9) examined the cross-sectional relation of FGF-23 to CAC in 162 individuals with CKD who were not receiving renal replacement therapy. In that study, patients in the highest tertile of FGF-23 demonstrated a 2.4-fold greater risk for prevalent CAC, although this association was no longer significant in multivariable-adjusted models (9). Roos *et al.* found no association between FGF-23 and CAC in 64 individuals who were suspected of having coronary artery disease and had normal kidney function (12).

To our knowledge, our study is the first to prospectively assess the relationship between FGF-23 and CAC progression. This is important because history of exposure to other risk factors may affect FGF-23 levels, kidney function, and subclinical atherosclerosis, causing confounding that may not be accounted for by single cross-sectional measurements.

Strengths of our study include the use of a well characterized, multiracial cohort with serial measurements of CAC. We also used both the Agatston method and volumetric scoring with mathematical transformation and found similar results. Several limitations deserve mention, including the relatively small sample size and the fact that FGF-23 was measured at only a single time point. Nonetheless, we believe this is the first report of the association of FGF-23 levels with repeated measures of CAC. Not all participants in our study had repeat CAC measurements. Interscan variability increases in parallel to CAC burden (30) and is an important source of bias in studies of CAC progression. We used mathematical transformation of volumetric scores to minimize the effect of interscan variability, a technique that has been well validated in other populations (16,31). Our study findings may not be generalizable to patients with less severe stages of CKD and therefore should be evaluated in other populations. The medication list was self-reported. As in many previous studies of FGF-23, blood samples in this study were stored without the use of protease inhibitors, which may have allowed for the degradation of FGF-23. However, the breakdown of FGF-23 would be likely to weaken, not strengthen, the relationship seen in our study. Finally, elevations in FGF-23 may cause physiologically relevant changes in 1,25 dihydroxyvitamin D levels. The fact that 1,25 dihydroxyvitamin D levels were not measured is an important limitation of this study (32).

In conclusion, elevated FGF-23 is an independent predictor of CAC progression in incident dialysis patients. Future investigations are needed to evaluate whether lowering FGF-23 is associated with decreased CAC progression and cardiovascular events.

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Disclosures

None.

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