Title:

A preliminary study of the potential role of FGF23 in coronary calcification in patients with suspected coronary artery disease

Hirofumi Masai[1], MD

Nobuhiko Joki[2], MD, PhD

Kaoru Sugi[1], MD, PhD

Masao Moroi[3], MD, PhD

[1] Division of Cardiovascular Medicine, and [2] Division of Nephrology, TOHO University Ohashi Medical Center

[3] Cardiology Division, National Center for Global Health and Medicine

Address for correspondence:

Nobuhiko Joki, MD, PhD, Associate Professor

Division of Nephrology, TOHO University Ohashi Medical Center, 2-17-6, Ohashi, Meguro-ku, Tokyo, 153-8515, Japan

Phone: +81-3-3468-1251; Fax: +81-3-3468-1269;

E mail: jokinobuhiko@gmail.com

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Abstract

Objective: The association of fibroblast growth factor 23 (FGF-23) with vascular disease in patients with preserved renal function is not well understood. The purpose of this study was to investigate the relationship of serum FGF-23 with coronary calcification in patients without chronic kidney disease and diabetes mellitus (DM).

Methods: A cross-sectional study was performed in 148 consecutive patients with suspected coronary artery disease who underwent 64-slice computed tomography coronary angiography for diagnosis of coronary artery disease. Patients with eGFR <60 mL/min/1.73m^2, proteinuria, or DM were excluded. Associations of coronary calcification (evaluated by Agatston score) were examined with classical risk factors and with inflammatory markers, adipocytokines and FGF-23.

Results: The median creatinine, eGFR and FGF-23 levels were 0.7 mg/dL, 74.6 mL/min/1.73m^2, and 26 pg/mL respectively. The strongest association was found between age and Agatston score ($r=0.367$, $p<0.001$) in univariate logistic regression analysis. No atherogenic risk factors, including inflammatory markers and adipocytokine levels, were associated with Agatston score. Among calcium/phosphate metabolism markers, FGF-23 showed a weak but significant correlation with Agatston score ($r=0.169$, $p=0.039$). In multivariate linear regression analysis, age and FGF-23 ($r=0.188$, $p=0.016$) were independently associated with the Agatston score.

Conclusion: Serum FGF-23 levels were associated with coronary calcification independently of classical risk factors and of adipocytokines and inflammatory markers in patients with preserved renal function. FGF-23 may also have a direct effect on progression of coronary calcification and further studies are required to examine this issue.

Key words: fibroblast growth factor 23, chronic kidney disease, arteriosclerosis, coronary artery calcification, computed tomography coronary angiography

1. Introduction

Quantification of coronary artery calcification (CAC) is widely understood to be of prognostic value, since patients with no CAC have very low rates of cardiac events [1-4]. CAC is a marker of subclinical atherosclerosis that is assessed by computed tomography and is strongly associated with the risk of future coronary heart disease and mortality [5]. Thus, it is important to detect early coronary calcification to avoid future cardiac events, but there is limited information on contributors to coronary calcification that could serve as surrogate markers in the general population.

Vascular calcification is often observed in patients with chronic kidney disease (CKD), especially in the advanced stage [6]. Calcium/phosphate (Ca/phosphate) disorder, and especially higher levels of blood phosphate, secondary to renal dysfunction has a central role in vascular calcification in these patients [6]. Fibroblast growth factor 23 (FGF-23) is a bone-derived hormone that has an important function in regulating circulating phosphate and vitamin D levels [7-11]. Circulating FGF-23 levels are elevated and independently associated with mortality in patients who are starting hemodialysis treatment [12]. Interestingly, several reports have shown that a higher FGF-23 level is also associated with vascular diseases such as carotid intima-media thickness [13], calcification of the aorta [14-16] and peripheral artery [16], and severity of angiographic coronary narrowing [17] in CKD patients. It is also of interest that the FGF-23 level starts to increase in the early phase of CKD (stage 1 or 2), even when the phosphate level is in the normal range [18].

Despite these findings, the association of FGF-23 with vascular disease in a population with preserved renal function remains unclear. The purpose of this study was to explore the relationship between the serum FGF-23 level and coronary calcification in patients with preserved renal function based on an estimated

glomerular filtration rate (eGFR) over 60 mL/min/1.73m^2.

2. Subjects and Methods

2.1. Study design and patients

We conducted a study with a cross-sectional design in 148 of 227 consecutive patients with suspected coronary heart disease who underwent computed tomography coronary angiography (CTCA) for diagnosis of coronary artery disease in our hospital from February 2010 to August 2011. The exclusion criteria were patients with eGFR <60 mL/min/1.73m^2, proteinuria, and diabetes (HbA1c > 5.8% or currently taking antidiabetic drugs). These criteria led to exclusion of 79 patients. The Agatston score for CAC was determined by CTCA and the associations of this score with clinical variables and blood chemistry data including FGF-23 were examined. The study protocol was approved by the Ethics Committee for Clinical Research of Toho University Ohashi Medical Center.

2.2. Data collection

Clinical information was recorded for all patients at the time of each CTCA examination. First, the patient was interviewed to obtain data on age, gender, smoking habits, previous hospitalizations, and history of hypertension, cardiac disease, cerebrovascular disease, and peripheral artery disease. Information about the use of oral medications was also collected. Blood pressure was recorded in the sitting position and a blood sample was collected just before injection of the contrast agent for CTCA. Blood samples were taken at least 4 hours after a meal. Body mass index (BMI) was calculated as weight (kg) / height^2 (m^2). eGFR was calculated using the new Japanese equation [19]: eGFR (mL/min/1.73 m^2) = 194×creatinine

$(Cr)^{-1.094} \times age^{-0.287}$ (×0.739 for women).

2.3. Serum and plasma biochemistry

All blood samples were drawn in the evening, just before CT angiograms were recorded. The subjects were instructed not to eat a meal within four hours before recording of the angiogram. Therefore, the blood samples were taken at least 4 hours after a meal. Plasma and serum samples were stored at -20°C immediately after blood sampling. All biochemical markers were measured within 2 weeks after sampling.

Blood chemistry parameters (low- and high-density lipoprotein cholesterol, triglyceride, creatinine, albumin, calcium, and inorganic phosphate, HbA1c, immunoreactive insulin) were measured by standard automated techniques in our hospital. The biologically active form of serum FGF-23 (intact FGF-23) was measured with a sandwich ELISA system (Kainos Laboratories Inc, Tokyo, Japan) [20]. Serum 1,25-$(OH)_2$-D_3 levels were measured using a TFB 1,25-dihydroxyvitamin D RIA kit (Immunodiagnostic Systems, Boldon, UK). Serum whole PTH levels were measured with a third-generation PTH assay (Scantibodies Laboratories, Santee, CA, USA). Serum levels of leptin, total adiponectin and TNF-α were measured using a RIA kit (Millipore Corporation, Billerica, MA, USA), a sandwich ELISA (Otsuka Pharmaceuticals, Tokyo, Japan), and a QantiGlo ELISA Kit (QTA00B; R&D Systems Inc, Minneapolis, MN, USA), respectively. FGF-23, 1,25-$(OH)_2$-D_3, whole-PTH, leptin, adiponectin, and TNF-α levels were all determined at Mitsubishi Chemical Medience Corporation (Tokyo, Japan).

2.3. Urine sampling

Of the 148 patients, 17 provided spot urine samples at the time of blood sampling.

Urine phosphate and Cr concentrations were measured by standard methods. The absolute urine phosphate concentration and this concentration adjusted by urine Cr were used for analysis. The adjusted urine phosphate concentration was calculated as urine phosphate (mg/dL) / urine Cr (mg/dL) (urine phosphate:creatinine ratio).

2.4. Computed tomography coronary angiography (CTCA)

The patients underwent 64-multislice CTCA with an Aquillion 64 system (Toshiba Medical Systems, Otawara, Japan) for diagnosis of coronary artery disease. Those with a resting heart rate >60 bpm received 1 mg/kg metoprolol orally 60 min before imaging, and all patients received 0.3 mg nitroglycerin sublingually 5 min before imaging. The settings in the 64 CTCA system were collimator 64 x 0.5 mm; detector pitch 9.8-11.2; pixel size 0.39 x 0.39 mm; gantry rotation time 350 ms; tube current 400 mA; and voltage 120 kV. Contrast agent (370 mgI/ml^{-1}; iopamidol, Bayer Schering Pharma) was injected at a rate of 0.06 mL/kg/s for the whole scan time plus 2 s, followed by 0.15 ml/kg of contrast media and 0.15 ml/kg of saline solution with a dual injector. Acquisition of CT data and electrocardiography (ECG) were started as soon as the signal density level in the ascending aorta reached a predefined threshold of 250 HU. The effective radiation dose was 15-18 mSv. Acquisition time was reduced to 175 ms by applying a half-scan algorithm (only data from a 180° gantry rotation were used for image reconstruction) in all patients. Raw data from the scans were reconstructed using a half-scan algorithm optimized for retrograde ECG-gated reconstruction. The end of the reconstruction period was set at the peak of the P wave on the monitoring ECG [21,22]. The reconstructed CT image data were transferred to a computer workstation (Ziostation, Amin, Tokyo, Japan) for post-processing and analysis. After visual inspection of the volume-rendered images, which depicted the gross

configuration of the lumen of the coronary artery, coronary artery plaques were inspected carefully on both the axial and curved multiplanar reformatted images, and were used to classify coronary lesions visually.

2.5. Assessment of coronary plaques

Images were evaluated with the optimal setting to detect plaques and outer vessel boundaries, using an average at a width representing 155% of the mean intensity within the lumen and at a level representing 65% of the mean intensity [23]. The coronary plaque area was measured by manual tracing because of the difference between the vessel area (inside area of the external elastic membrane) and the lumen area at the site of maximal lumen narrowing, as observed on cross-sectional 64-slice CTCA images. A lipid-rich plaque area was defined as an area of mean CT density <50 HU, which was automatically traced and measured on the workstation (Ziostation). An area with a mean CT density <50 HU is thought to be a lipid-rich plaque on IVUS images [24]. Agatston scores for CAC were determined using software for evaluation of calcification.

2.6. Statistical analysis

Data are expressed as the median and interquartile range. Pearson univariate regression analysis was used to evaluate the association of clinical factors with Agatston scores. Dummy variables were used for gender (0 for female, 1 for male), smoking history (0 for negative, 1 for positive), and use of each medication (0 for not used, 1 for used). In all analyses, $P<0.05$ was considered significant. All analyses were performed using StatView for Windows v. 5.0 (SAS Institute, Cary, NC).

3. Results

3.1. Patient characteristics

The 148 subjects had a median age of 65.5 years old (range: 27 to 84 years old), about half were male, and about half were smokers. Median BMI was 23.1, which is similar to the value found in epidemiological studies of the general population in Japan. Medical history, medications and biochemical data are shown in Table 1. The median Cr level and eGFR were 0.7 mg/dL and 74.6 mL/min/1.73m^2, respectively, indicating preserved renal function. The median level of FGF-23 was 26 pg/mL, which is almost the same as that in the general Japanese population [25]. Log transformed FGF-23 is also shown in Table 1 and histograms of absolute and Log transformed FGF-23 levels are shown in Figure 1. Absolute FGF-23 had a near normal distribution. The median calcium, phosphate, whole-PTH, and 1,25-$(OH)_2$-D_3 levels were 9.5 mg/dL, 3.7 mg/dL, 36.6 pg/mL, and 56 pg/mL respectively, all of which were within the normal ranges. Lipid profile, inflammation markers, and adipocytokines are also shown in Table 1. Coronary characteristics from the CT angiogram are shown in Table 2. The median Agatston score was 11.5 (range, 0 to 1445) and 15.5% of the subjects had significant coronary narrowing.

3.2. Factors associated with FGF-23

FGF-23 levels were significantly correlated with phosphate levels (r=0.18, p=0.028) and significantly inversely related to eGFR (r=-0.201, p=0.014) (Table 3). There was no association of FGF23 with 1,25-$(OH)_2$-D_3 levels (r=-0.132, p=0.109). FGF-23 was also significantly negatively correlated with adiponectin levels (r=-0.182, p=0.043), but not with other lipid parameters or inflammatory markers. There was also no association between serum FGF23 and coronary plaque area (data not shown).

In urine data, FGF-23 was strongly correlated with absolute urine phosphate concentration (r=0.564, p=0.018). No association was found between FGF-23 and the urine phosphate:creatinine ratio.

3.3. Factors associated with coronary calcification

In univariate regression analysis (Table 4), Agatston scores (reflecting coronary calcification) were significantly associated with age (r=0.367, p<0.001) and HbA1c (r=0.216, p=0.008). Among Ca/phosphate metabolism markers, FGF-23 levels showed a weak but significant correlation with Agatston score (r=0.169, p=0.039) (Figure 2). An inverse relationship was found between eGFR and Agatston scores (r=-0.166, p=0.043). There was a tendency towards a negative association of Agatston scores with 1,25-(OH)2-D3 levels, but this association failed to reach the level of statistical significance (r=-0.156, p=0.058). Agatston scores showed no relationship with absolute or adjusted urine phosphate concentrations.

3.4. Independent factors associated with coronary calcification

Based on the results of univariate analysis, age, HbA1c, eGFR, 1,25-$(OH)_2$-D_3 and FGF-23 were used in multivariate linear regression analysis of the Agatston score. As shown in Table 5, age, 1,25-$(OH)_2$-D_3 and FGF-23 were independently associated with the Agatston score.

4. Discussion

It is well established that the presence of coronary calcification is a good predictor of future cardiac events in patients with and without CKD. However it remains unclear whether mineral metabolism plays a role in the progression of vascular calcification in

patients with preserved renal function. Our study showed that higher serum FGF-23 is associated with coronary calcification independently of age and renal function in patients with suspected coronary artery disease and without kidney disease. FGF-23 regulates circulating phosphate, active vitamin D, and PTH levels, all of which also regulate FGF-23 production from bone. Thus our findings imply that Ca/phosphate metabolism plays a role in promoting coronary calcification, even in patients with preserved renal function. However, the cross sectional design of the study does not permit an assessment of the causality of FGF-23 in progression of coronary calcification in these patients.

Vascular calcification is often observed in older persons and in patients with diabetes or end-stage kidney disease (ESKD). The pathophysiology of vascular calcification in ESKD has been widely investigated. Ca/phosphate metabolic disorder secondary to impaired renal function plays a central role in initiation or progression of vascular calcification. In particular, a high phosphate concentration has a crucial impact on vascular calcification and is an important therapeutic target in CKD patients [26-28]. Recent studies have demonstrated the importance of extracellular phosphate as a substrate for hydroxyapatite formation and as an inducer of gene expression programs that facilitate calcification [29-30]. It is of interest that higher FGF-23, which promotes renal phosphate excretion, is associated with higher mortality in patients with ESKD [12]. Parker et al. also found that higher FGF-23 levels were associated with mortality and composite cardiovascular events in 833 outpatients with coronary artery disease and an eGFR of 70 to 80 mL/min/1.73m^2 [31].

In our study, serum phosphate concentrations were normal in most of the cohort with preserved renal function, as expected, and had no association with vascular calcification. At this level of renal function, the serum phosphate level may be

maintained within a normal range by a compensatory increase of FGF-23, even if the time-averaged phosphate level is high. Our results are consistent with this theory. Thus, FGF-23 may be a surrogate marker for the time-averaged serum phosphate level and might be expected to have a close association with coronary calcification in patients with preserved renal function. We failed to observe an association between urinary phosphate and coronary calcification. However, the number of patients was limited. Examination of a possible association of urinary phosphate excretion with coronary calcification in a larger patient sample should allow a definitive answer to this question.

Another potential explanation of the link between high FGF-23 and an increased risk of vascular disease is the connection of FGF-23 with lipid metabolism. In an evaluation of the relationships between FGF-23 and metabolic cardiovascular risk factors in two independent community-based, cross-sectional cohorts of elderly individuals, Mirza et al. [32] found significant correlations of serum FGF-23 levels with higher BMI, larger waist circumference, elevated triglycerides (TG), and lower HDL-cholesterol (HDL-C). Thus, FGF-23 may exert an indirect effect on lipid metabolism, and as a consequence may promote vascular disease. Indeed, elevated TG [33] and lower HDL-C [34] are closely associated with coronary calcification, and we also found a correlation of FGF-23 with TG, but this did not reach a significant level in our subjects.

A third possible mechanism underlying the link between FGF-23 and vascular disease may involve a connection between adipocytokines and FGF-23. Tsuji et al. recently showed that leptin, a peptide hormone produced by adipocytes and related to total body fat, directly stimulates FGF-23 expression in bone in leptin-deficient mice [35]. FGF-23 levels are also inversely related to those of adiponectin, a hormone that

protects against endothelial damage [36], and elevated leptin and lower adiponectin are well known contributors to vascular calcification in humans [37]. An inverse relationship of FGF-23 with the level of adiponectin was also observed in our study.

A direct effect of FGF-23 on coronary calcification is a final potential explanation for our findings. To our knowledge, there are no studies that support a direct toxic influence of FGF-23 on vascular damage. However, recently, it has been proven that FGF-23 directly affects left ventricular hypertrophy in an animal model [38]. Therefore, it is possible that FGF-23 may have a direct role in progression of vascular damage.

It is of interest that lower 1,25-$(OH)_2$-D_3 levels were associated with coronary calcification, independent of age, renal function, and FGF-23. This finding is supported by evidence that not only a high level, but also a low level of 1,25-$(OH)_2$-D_3 was associated with significantly greater vascular calcification in pediatric dialysis patients [39]. The potential mechanism may involve the association of low 1,25-$(OH)_2$-D_3 levels with higher CRP concentrations, which would promote vascular calcification [40].

There are several limitations in the current study. As mentioned above, based on our study design, elevated FGF-23 can only be defined as a marker of coronary calcification in patients with preserved renal function. Evidence for a causal relationship and clarification of the mechanism of this unique finding will require a longitudinal study of the effect of changes in FGF-23 on vascular calcification. Second, we did not collect urine data, especially for the urine phosphate level, which is mainly regulated by FGF-23. These data may permit development of a hypothesis that phosphate load plays a role in promoting coronary calcification. Third, the number of patients was small and a further investigation in a larger sample size is needed.

5. Conclusion

Serum FGF-23 levels were associated with coronary calcification independently of classical risk factors and also of adipocytokines and inflammatory markers in patients with preserved renal function. A direct effect of FGF-23 on progression of coronary calcification cannot be excluded and a further study is required to examine this issue.

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Conflict of Interest

None of the authors have a conflict of interest regarding the work in the study. Funding was provided from institutional sources only.

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Figure legends

Figure 1

Histograms of absolute and Log transformed FGF-23 levels. Absolute FGF-23 had a near normal distribution.

Figure 2

A weak but significant correlation of serum FGF23 level with Agatston score was observed (r=0.169, p=0.039) by Pearson univariate regression analysis.

Table 1: Characteristics of patients with eGFR >60 ml/min/1.73m^2 without proteinuria [#]

Age, years	65.5 (55, 72)
Male, %	81(54.7)
Body mass index, kg/m2	23.1 (21.2, 25.5)
Smoker, %	73(49.3)
Systolic blood pressure, mmHg	130 (120, 140)
Diastolic blood pressure, mmHg	78 (70, 82)
Hypertension, %	83(56.1)
Dyslipidemia, %	87(58.7)
Angina pectoris, %	5(3.3)
Myocardial infarction, %	3(2)
Statin, %	42(28.3)
β-blocker, %	9(6)
RAS, %	38(25.6)
CCB, %	51(34.4)
Diuretics, %	8(5.4)
Antiplatelet, %	27(18.2)
LDL-C, mg/dl	114 (92, 131)
HDL-C, mg/dl	54 (43, 64)
Triglyceride, mg/dl	117 (79, 182)
HbA1c, %	5.2 (5, 5.5)
IRI, μU/ml	4.3 (3, 8.3)
HOMA index	1.04 (0.88, 1.91)

Serum creatinine, mg/dl	0.7 (0.62, 0.83)
eGFR, ml/min/1.73m2	74.6 (66.7, 83.5)
C-reactive protein, mg/dl	0.046 (0.029, 0.298)
Calcium, mg/dl	9.5 (9.3, 9.8)
Phosphorus, mg/dl	3.7 (3.3, 4.1)
Calcium-phosphorus product	35.5 (31.6, 38.8)
Serum FGF-23, pg/ml	26 (21, 33)
Log Serum FGF-23	1.41(1.32, 1.51)
Whole PTH, pg/ml	36.6 (29.3, 47.4)
1,25-(OH)2-D3, pg/ml	56 (45, 76)
Leptin, ng/ml	7.1 (4.4, 9.9)
TNF-α, pg/ml	0.99 (0.79, 1.29)
Adiponectin, μg/ml	7.6 (5.6, 10.7)
Agatston score	11.5 (0, 193)
Urine creatinine, mg/dL*	119.4(70.8, 140.2)
Urine phosphate, mg/dL*	43.9(28.6, 58)
Urine phosphate, mg/dl (corrected for creatinine)*	0.44(0.29, 0.52)

Data are presented as n (%) or median (interquartile range).

The range of eGFR was 60-112 mL/min/1.73^2

* Analysis performed in 17 patients

Table 2: Coronary characteristics

	mean	median
Agatston score	149±276	11.5
Calcified plaques (%)	54.7	
Non-calcified plaques (%)	23.6	
Significant stenosis (%)	15.5	

Table 3:

Univariate linear regression analysis for factors associated with serum FGF-23

Variables	r	95%CI	P value
BMI	0.038	-0.397 - 0.641	0.642
Triglyceride	0.112	0.005 - 0.026	0.173
LDL	0.025	-0.045 - 0.061	0.760
HDL	-0.073	-0.184 - 0.07	0.38
L/H	0.075	-1.067 - 2.867	0.367
Leptin	0.132	-0.055 - 0.474	0.119
Adiponectin	-0.182	-0.729 - -0.011	0.043
TNF-α	-0.043	-1.297 - 0.899	0.719
C-reactive protein	-0.032	-18.708 - 13.165	0.731
eGFR	-0.201	-0.33 - -0.037	0.014
Calcium	0.037	-3.414 - 5.417	0.654
Phosphorus	0.18	0.285 - 5.165	0.028
1,25-(OH)2-D3	-0.132	-0.142 - 0.015	0.109
whole PTH	0.033	-0.077 - 0.116	0.691
Spot urine phosphate, mg/dl*	0.564	0.037 - 0.348	0.018
Spot urine phosphate, mg/dl correct creatinine*	0.011	-41.06 - 42.758	0.966

* Analysis performed in 17 patients

Table 4:

Univariate linear regression analysis for factors associated with Agatston score

Variables	r	95%CI	P value
Age	0.367	4.787 - 11.564	<0.001
HbA1c	0.216	46.246 - 306.901	0.008
LDL-C	0.008	-1.319 - 1.458	0.921
HDL-C	-0.087	- 5.088 - 1.552	0.294
Triglyceride	-0.025	-0.466 - 0.343	0.764
SBP	0.065	- 1.827 - 4.275	0.429
DBP	-0.047	- 6.044 - 3.339	0.569
eGFR	-0.166	- 7.831 - -0.115	0.043
Creatinine	0.056	-215.125 - 442.188	0.495
BMI	0.05	-9.371 - 17.748	0.542
C-reactive protein	0.076	-234.134 - 558.593	0.419
TNF-α	0.071	- 23.337 - 42.845	0.558
HOMA index	0.037	- 5.058 - 8.015	0.655
Adiponectin	0.004	- 9.565 - 9.99	0.965
Leptin	0.055	- 4.477 - 8.908	0.513
Calcium	-0.129	-205.787 - 23.24	0.117
Phosphorus	0.067	- 31.755 - 76.126	0.417
Whole PTH	0.134	- 0.434 - 4.556	0.104
Ca×P	0.04	- 4.005 - 6.637	0.625
1,25-(OH)2-D3	-0.156	- 4.006 - 0.69	0.058
Serum FGF-23	0.169	0.216 - 8.642	0.039

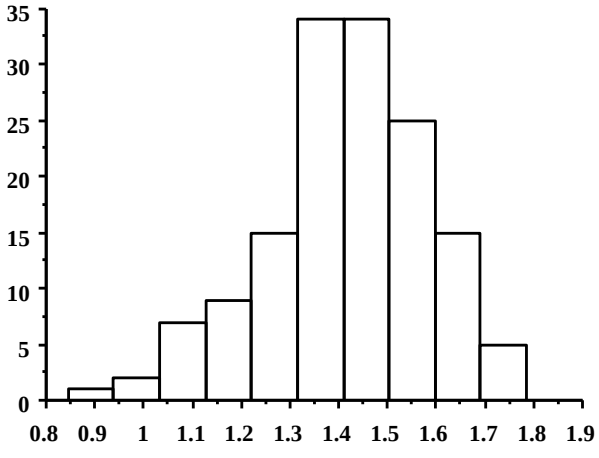
Log FGF-23	0.163	1.575 - 525.178	0.048
Spot urine phosphate, mg/dl*	-0.27	-6.183 - 2.012	0.295
Spot urine phosphate, mg/dl correct creatinine*	-0.27	-1380 - 447	0.294

* Analysis performed in 17 patients

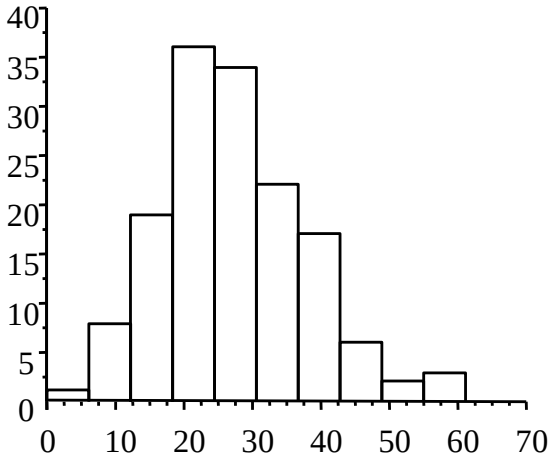
Table 5: Multiple linear regression analysis for factors associated with Agatston score

Variables	β	95%CI	P value
Age	0.376	4.82 - 11.948	<0.001
Serum FGF-23	0.188	0.922 - 8.913	0.016
eGFR	0.008	-3.589 - 3.970	0.92
1,25-(OH)2-D3	-0.175	-4.080 - -0.334	0.021
HbA1c	0.129	-18.051 - 228.987	0.093
R^2	0.224		<0.001

Figure 1

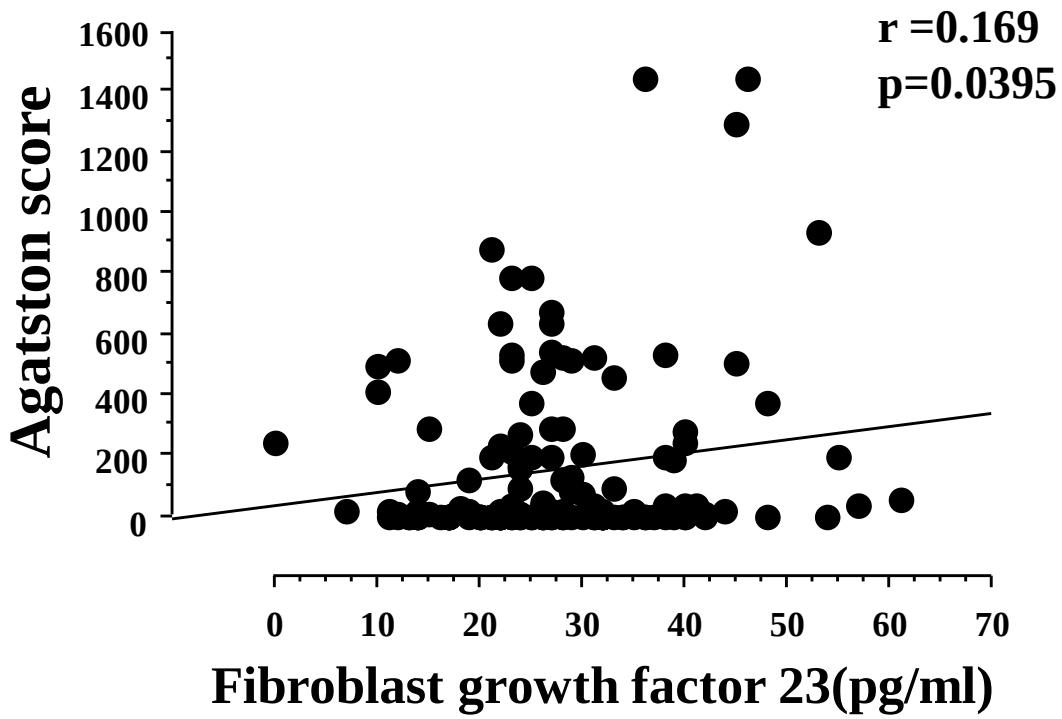


Log Serum FGF-23



Serum FGF-23

Figure 2


r =0.169
p=0.0395
Agatston score
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
70
Fibroblast growth factor 23(pg/ml)