

Original Article

Associations of serum fibroblast growth factor 23 with dyslipidemia and carotid atherosclerosis in chronic kidney disease stages 3-5D

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Abstract: *Background and aim:* Fibroblast growth factor 23 (FGF23) has been associated with mineral disorders and poor outcomes in patients with chronic kidney disease (CKD). Since FGF23 shares structural similarities with FGF19 subfamily members, which play a role in lipid metabolism, we examined the relationship of FGF23 with lipid metabolism and carotid atherosclerosis in CKD patients. *Material and methods:* A sample of 180 patients with CKD stages 3-5D, and 50 controls were recruited. Baseline serum intact FGF23 levels were measured and the associations of FGF23 with total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and body mass index (BMI) were explored. Carotid atherosclerosis was assessed using ultrasound. *Results:* In CKD patients, a per SD increase in logFGF23 was associated with lower TC ($\beta=-0.37$; $P=0.041$), LDL-C ($\beta=-0.33$; $P=0.040$), HDL-C ($\beta=-0.14$; $P=0.027$), BMI ($\beta=-1.15$; $P=0.030$), increased carotid intima media thickness (CIMT) ($\beta=0.03$; $P=0.037$), higher risk of carotid plaque (OR=3.74; $P=0.012$) and atherosclerosis (OR=3.68; $P=0.015$), after adjustment for age, sex, cardiovascular risk factors, albumin, mineral metabolism markers, CKD stage, lipid lowering drug use, and vitamin D analogues. Receiver operating characteristic (ROC) curves for multivariate regression models to predict carotid atherosclerosis showed that the full model including serum FGF23 had the highest model discrimination (AUC=0.940, 95% CI: 0.900, 0.980, $P<0.001$). *Conclusions:* Serum FGF23 is closely associated with dyslipidemia and could become a useful marker for the prediction of carotid atherosclerosis in patients with CKD stages 3-5D.

Keywords: Fibroblast growth factor 23, chronic kidney disease, dyslipidemia, carotid atherosclerosis

Introduction

Fibroblast growth factor 23 (FGF23), a protein primarily secreted by osseous tissue, stimulates urinary phosphate excretion and reduces intestinal phosphate absorption by inhibiting the synthesis of 1,25-dihydroxyvitamin D in order to maintain the balance of phosphate metabolism in chronic kidney disease (CKD) patients [1, 2]. FGF23 has been considered one of the earliest biomarkers in chronic kidney disease-mineral and bone disorder (CKD-MBD) [3-5]. FGF23 has also been reported to be related to a high risk of cardiovascular diseases (CVD) such as atherosclerosis, left ventricular hypertrophy, vascular calcification, vascular dysfunction, and increased mortality [6-10]. However, the specific underlying pathogenesis has not been elucidated.

Recently, FGF23 was found to have an association with factors other than mineral metabolism, as it belongs to the FGF19 subfamily and shares structural and biological similarities with the other family members (FGF19 and FGF21) that are involved in lipid and glucose metabolic homeostasis [11]. Studies have demonstrated that FGF23 levels correlate with the development of dyslipidemia in elderly individuals [12] and in hemodialysis patients [13, 14]. Atherosclerosis is one of the cardiovascular pathologies that contributes to increased mortality in CKD patients. Lipid abnormalities, in addition to the mineral and bone disorders, frequently lead to accelerated atherosclerosis [15], suggesting that the association of FGF23 with CVD and mortality may be in part mediated by traditional metabolic factors.

Associations of FGF23 with dyslipidemia and atherosclerosis in CKD

To the best of our knowledge, the relationship between FGF23 and dyslipidemia has rarely been reported in the CKD population, particularly in non-dialysis and peritoneal dialysis patients. Thus, we carried out this cross-sectional study aiming to elucidate the association of FGF23 with dyslipidemia, body mass index (BMI), and carotid atherosclerosis in patients with CKD stages 3-5D.

Material and methods

Patient selection

We recruited 180 patients with CKD stages 3-5D including non-dialysis (ND), peritoneal dialysis (PD), and hemodialysis (HD) patients between April 2010 and March 2014. CKD Stages 3-5 were defined by the Modification of Diet in Renal Disease estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m². All dialysis patients were established for at least 6 months. Average Kt/V_{urea} was 1.73 ± 0.38 in HD (daily) and 1.96 ± 0.48 in PD patients (weekly). We also enrolled 50 apparently healthy individuals as controls from the medical examination center. The study was carried out in accordance with the tenets of the Helsinki Declaration and was approved by the Ethics Committee of Renji Hospital. All of the participants gave informed consent.

The exclusion criteria were (1) pregnancy; (2) psychosis; (3) active infection or having received antibiotics over the last month; (4) surgery or trauma over the last month; (5) familial hyperlipidemia; (6) acute cardiovascular event (such as acute coronary syndrome, new-onset arrhythmia, heart failure, stroke or arterial dissection) over the last month; (7) malignancy and severe malabsorption.

Clinical information

The following baseline demographic and clinical data were collected: age, sex, history of hypertension, diabetes, smoking, current medication use and cardiovascular comorbidity. Prevalent CVD was defined as a history of acute coronary syndrome, congestive heart failure, major stroke, carotid atherosclerosis and peripheral vascular disease [16]. BMI was calculated as weight (kg)/height (m)². Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after 10 minutes of

rest. The mean arterial blood pressure (MABP) was calculated as $DBP + 1/3 (SBP - DBP)$.

Laboratory measurements

Fasting serum samples were collected under standard conditions at the baseline visit. The samples were immediately centrifuged at 4000 r.p.m. for 10 minutes at room temperature. Supernatants were stored in aliquots at -80°C for further use. Hemoglobin (Hgb), serum creatinine (Scr), blood urea nitrogen (BUN), albumin, pre-albumin, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), fasting blood glucose (FBG), alkaline phosphatase (AKP), calcium (Ca), phosphorus (P), intact parathyroid hormone (iPTH), and high sensitive C-reactive protein (hsCRP) levels were measured using conventional laboratory techniques. Corrected serum Ca was applied and calculated using the formula: corrected Ca = measured Ca + $(1 - 0.025 \times \text{albumin})$. Serum 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] levels were measured using enzyme-linked immunosorbent assay (ELISA) Kits (IDS Ltd., Tyne & Wear, UK). FGF23 levels were measured from serum samples by using the human intact FGF23 ELISA kit (Kainos Laboratories Inc., Tokyo, Japan). The kit recognizes the sites of antigen binding with the amino terminal and carboxyl terminal of FGF23 by using two specific mouse anti-human monoclonal antibodies, with a sensitivity of 3.0 pg/mL and an intra- and inter-assay CVs of $<5.0\%$.

Carotid ultrasound

Patients were examined using a B-mode ultrasound scanner (HP-HX Color Doppler Ultrasound System, Hewlett-Packard Company, Palo Alto, CA, USA) equipped with a 10-MHz linear probe. Carotid atherosclerotic plaque was defined as a local intima that was thicker than 1 mm and more than twice the thickness of the neighboring sites. Carotid intima media thickness (CIMT) was measured at the far wall of the common carotid artery, 2-4 cm proximal to the bifurcation. CIMT was defined as the distance between the leading edges of the lumen interface and the media-adventitia interface of the far wall. Carotid atherosclerosis was defined as increased CIMT (>1.0 mm) or presence of a plaque [17].

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Table 1. Baseline characteristics and laboratory parameters

	Control n=50	ND n=60	PD n=50	HD n=70	P-value
Age (year)	57.32±8.56	56.97±12.88	55.60±13.21	58.03±12.57	NS
Male, n (%)	24 (48%)	36 (60%)	28 (56%)	37 (53%)	NS
BMI (kg/m ²)		23.33±3.40	22.60±3.22	21.51±3.31**	<0.01
CVD, n (%)		21 (35%)	27 (54%)	36 (51%)	NS
Smokers, n (%)		15 (25%)	10 (20%)	12 (17%)	NS
Vitamin D analogues		1 (2%)	14 (28%)*	37 (53%)* ^Δ	<0.01
Lipid lowering drugs		6 (10%)	12 (24%)*	22 (31%)*	<0.05
Diabetes, n (%)		12 (20%)	10 (20%)	10 (14%)	NS
MABP (mmHg)		102.48±9.07	102.75±10.16	101.02±13.86	NS
Dialysis vintage (mon)			31.50 (17.00, 64.00)	56.00 (25.00, 85.00)	NS
FBG (mmol/L)	5.41±0.62	5.02±1.30	5.30±2.03	5.43±0.92	NS
Hgb (g/dL)	140.36±13.92	92.68±17.64 ^{▲▲}	110.04±15.12 ^{▲▲**}	112.17±17.14 ^{**}	<0.01
Ca (mmol/L)		2.08±0.24	2.12±0.22	2.34±0.25 ^{**ΔΔ}	<0.01
P (mmol/L)		1.61±0.44	1.68±0.43	1.70±0.52*	<0.05
Ca×P (mg ² /dL ²)		41.49±11.36	44.48±12.69	48.93±16.41*	<0.05
PTH (pg/mL)		218.00 (115.00, 330.00)	342.00 (131.75, 541.75)	378.00 (167.00, 643.00)*	<0.05
AKP (U/L)	76.00 (64.00, 89.50)	79.20 (61.60, 108.90)	115.50 (90.00, 150.00) ^{▲▲**}	162.00 (110.00, 236.00) ^{▲▲***ΔΔ}	<0.01
TC (mmol/L)	4.42±0.71	4.71±0.96	4.78±1.01	4.19±0.98 ^{**ΔΔ}	<0.01
TG (mmol/L)	1.50±0.81	1.62±0.81	2.01±1.83	2.02±1.38	NS
LDL-C (mmol/L)	2.65±0.63	2.92±0.82	2.81±1.04	2.11±0.80 ^{▲**ΔΔ}	<0.01
HDL-C (mmol/L)	1.44±0.31	1.21±0.29 [▲]	1.15±0.38 ^{▲▲}	1.16±0.41 ^{▲▲}	<0.05
TG/HDL-C ratio	0.91 (0.68, 1.38)	1.18 (0.89, 1.98) [▲]	1.37 (0.81, 2.73) [▲]	1.54 (0.77, 2.49) ^{▲▲}	<0.05
Albumin (g/L)	45.72±2.20	40.46±3.01 ^{▲▲}	36.75±4.78 ^{▲▲*}	38.71±5.01 ^{▲▲}	<0.01
Pre-albumin (mg/L)	290.46±24.97	325.00±62.14	349.14±68.14 ^{▲▲}	352.34±82.25 ^{▲▲}	<0.01
HsCRP (mg/L)		1.72 (0.97, 3.62)	2.23 (1.32, 4.12)	2.57 (1.00, 4.81)	NS
25(OH)D (ng/mL)	17.42 (14.42, 22.64)	14.37 (10.47, 17.34) ^{▲▲}	14.90 (11.68, 19.60) [▲]	15.73 (13.34, 20.05)	<0.01
1,25(OH) ₂ D (pmol/L)	43.99 (38.78, 51.65)	33.39 (29.00, 42.57) ^{▲▲}	28.78 (19.90, 42.73) ^{▲▲}	27.15 (14.68, 44.83) ^{▲▲}	<0.01
FGF23 (pg/mL)	27.17 (21.63, 51.20)	196.46 (83.09, 355.02) ^{▲▲}	1625.80 (602.83, 7521.78) ^{▲▲**}	2507.89 (1191.35, 7400.41) ^{▲▲**}	<0.01

ND, non-dialysis group; PD, peritoneal dialysis group; HD, hemodialysis group; BMI, body mass index; CVD, cardiovascular disease history; MABP, mean arterial blood pressure; FBG, fasting blood glucose; Hgb, Hemoglobin; Ca, calcium; P, phosphorus; PTH, parathyroid hormone; AKP, alkaline phosphatase; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; hsCRP, high sensitive C-reactive protein; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D. Compared with control, ^{▲▲}P<0.01, [▲]P<0.05. Compared with ND, ^{**}P<0.01, ^{*}P<0.05. Compared with PD, ^{ΔΔ}P<0.01, ^ΔP<0.05.

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Table 2. Correlations between logFGF23 and clinical parameters in CKD patients

Parameter	Pearson's r	P
Age	0.11	NS
BMI	-0.15	0.045
MABP	0.01	NS
FBG	0.04	NS
Hgb	0.10	NS
Ca	0.37	<0.001
P	0.57	<0.001
Ca×P	0.64	<0.001
logPTH	0.35	<0.001
logAKP	0.12	NS
TC	-0.17	0.027
TG	0.11	NS
LDL-C	-0.21	0.006
HDL-C	-0.19	0.013
logTG/HDL-C	0.13	NS
Albumin	-0.10	NS
Pre-albumin	0.18	0.035
loghsCRP	0.26	<0.001
log25(OH)D	0.12	NS
log1,25(OH) ₂ D	-0.18	0.021

BMI, body mass index; MABP, mean arterial blood pressure; FBG, fasting blood glucose; Hgb, Hemoglobin; Ca, calcium; P, phosphorus; PTH, parathyroid hormone; AKP, alkaline phosphatase; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; hsCRP, high sensitive C-reactive protein; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D.

Statistical analyses

Statistical analyses were performed using SPSS 23.0. The level of significance was set at $P < 0.05$ for 2-sided tests. Variables were expressed as the mean \pm SD if normally distributed or the median (interquartile range) for non-normally distributed data. Values of non-normal distribution were transformed to a log base of 10 for the univariate correlation and regression analyses. Normally distributed data were compared using t-test for two groups and oneway ANOVA for multiple groups. Non-normally distributed data were compared using Mann-Whitney U-test for two groups and Kruskal-Wallis rank sum tests for multiple groups. Categorical data were compared using chi-square test. Univariate correlation was analyzed using Pearson's correlation test.

Multiple regression analysis was used to assess the relationship between one dependent variable and multiple independent variables. Binary logistic regression was used to analyze the risk factors for carotid plaque and carotid atherosclerosis. Several models were used in multivariate analyses. The covariates included in the models were the important clinical variables, cardiovascular risk factors, parameters correlated with mineral metabolism, and factors thought to be biologically plausible.

Results

Baseline characteristics and laboratory measurements

The patients' baseline characteristics and laboratory parameters of each group are presented in **Table 1**. The underlying causes for CKD were chronic glomerulonephritis ($n=88$, 49.0%), diabetic kidney disease ($n=26$, 14.3%), hypertensive nephrosclerosis ($n=22$, 12.1%), polycystic kidney disease ($n=4$, 1.9%), obstructive nephropathy ($n=3$, 1.6%), and other causes or unknown conditions ($n=37$, 21.1%). There were significant differences in the levels of BMI, Hgb, Ca, P, Ca×P, PTH, AKP, TC, LDL-C, HDL-C, TG/HDL-C ratio, albumin, pre-albumin, 25(OH)D, and 1,25(OH)₂D among the different groups. As for drug use, the PD and HD groups had significantly higher usage of vitamin D analogues and lipid lowering drugs than the ND group ($P < 0.05$).

FGF23 levels and associated factors

The median FGF23 levels of the control, ND, PD, and HD groups were 27.17 (21.63, 51.20) pg/mL, 196.46 (83.09, 355.02) pg/mL, 1625.80 (602.83, 7521.78) pg/mL and 2507.89 (1191.35, 7400.41) pg/mL, respectively. The FGF23 levels in the ND group were significantly higher than those of the controls ($P < 0.01$). The levels of FGF23 were significantly higher in dialyzed patients than in non-dialysis patients ($P < 0.01$, **Table 1**).

Pearson's analysis showed that the logFGF23 was positively correlated with Ca ($r=0.37$, $P < 0.001$), P ($r=0.57$, $P < 0.001$), Ca×P ($r=0.64$, $P < 0.001$), logPTH ($r=0.35$, $P < 0.001$), pre-albumin ($r=0.18$, $P=0.035$), and loghsCRP ($r=0.26$, $P < 0.001$), and negatively correlated with log1,25(OH)₂D ($r=-0.18$, $P=0.021$), TC ($r=-0.17$,

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Table 3. Associations of FGF23 with components of the lipid profile and BMI

Independent variable: log ₁₀ FGF23		Crude	Model 1	Model 2
TC	β (95% CI)	-0.20 (-0.38, -0.02)	-0.24 (-0.42, -0.06)	-0.37 (-0.71, -0.01)
	t	-2.24	-2.68	-2.07
	P	0.027	0.008	0.041
LDL-C	β (95% CI)	-0.23 (-0.39, -0.06)	-0.24 (-0.40, -0.07)	-0.33 (-0.64, -0.01)
	t	-2.76	-2.83	-2.08
	P	0.006	0.005	0.040
HDL-C	β (95% CI)	-0.08 (-0.15, -0.02)	-0.10 (-0.16, -0.03)	-0.14 (-0.25, -0.01)
	t	-2.50	-2.78	-2.23
	P	0.013	0.006	0.027
TG	β (95% CI)	0.12 (-0.12, 0.36)	0.09 (-0.15, 0.33)	0.31 (-0.10, 0.75)
	t	1.01	0.73	1.53
	P	0.314	0.468	0.135
log ₁₀ TG/HDL-C	β (95% CI)	0.03 (-0.03, 0.09)	0.03 (-0.03, 0.09)	0.12 (0.01, 0.22)
	t	0.99	0.86	1.43
	P	0.385	0.421	0.167
BMI	β (95% CI)	-0.60 (-1.29, -0.08)	-0.60 (-1.20, -0.01)	-1.15 (-2.16, -0.12)
	t	-2.02	-1.98	-2.20
	P	0.045	0.049	0.030

Model 1: adjusted for age and sex. Model 2: adjusted for Model 1 plus hypertension, diabetes, history of CVD, smoking, serum albumin, Ca, P, PTH, 25(OH)D, 1,25(OH)₂D, hsCRP, CKD stage, administration of lipid lowering drugs and vitamin D analogues.

P=0.027), LDL-C ($r=-0.21$, $P=0.006$), HDL-C ($r=-0.19$, $P=0.013$), and BMI ($r=-0.15$, $P=0.045$) in CKD patients (**Table 2**).

FGF23 and dyslipidemia

The crude and multivariate adjusted associations between serum FGF23 levels and components of the lipid profile in CKD patients are presented in **Table 3**.

In the crude model, significantly negative relationships were found between FGF23 levels and TC ($\beta=-0.20$, 95% CI: -0.38, -0.02, $P=0.027$), LDL-C ($\beta=-0.23$, 95% CI: -0.39, -0.06, $P=0.006$), and HDL-C ($\beta=-0.08$, 95% CI: -0.15, -0.02, $P=0.013$) levels.

Similarly, higher FGF23 levels were associated with lower TC ($\beta=-0.24$, 95% CI: -0.42, -0.06, $P=0.008$), LDL-C ($\beta=-0.24$, 95% CI: -0.40, -0.07, $P=0.005$), and HDL-C ($\beta=-0.10$, 95% CI: -0.16, -0.03, $P=0.006$) levels in model 1.

In the fully adjusted model (model 2), the correlations remained with TC ($\beta=-0.37$, 95% CI: -0.71, -0.01, $P=0.041$), LDL-C ($\beta=-0.33$, 95% CI: -0.64, -0.01, $P=0.040$), and HDL-C ($\beta=-0.14$, 95% CI: -0.25, -0.01, $P=0.027$) levels.

In all three models, no significant relationship was observed between FGF23 levels and either TG level or the TG/HDL-C ratio.

FGF23 and BMI

The BMI in the HD group was significantly lower than in the ND group ($P<0.01$). Linear regression analysis revealed a negative correlation between FGF23 level and BMI in the crude model ($\beta=-0.60$, 95% CI: -1.29, -0.08, $P=0.045$). After adjusting for age and sex (model 1), the association still remained ($\beta=-0.60$, 95% CI: -1.20, -0.01, $P=0.049$). Further adjustment in model 2 also indicated a statistically significant relation between higher FGF23 level and decreased BMI ($\beta=-1.15$, 95% CI: -2.16, -0.12, $P=0.030$. **Table 3**).

FGF23 and carotid atherosclerosis

Using ultrasound, we found 19 (32%), 29 (58%), and 48 (68%) patients with carotid plaque in ND, PD and HD group, respectively. The median FGF23 levels of the patients with zero plaque ($n=84$), 1 plaque ($n=35$), 2 plaques ($n=33$), and ≥ 3 plaques ($n=28$) were 487.48 (128.28, 3760.75) pg/mL, 636.65 (126.63, 1563.27) pg/mL, 871.11 (217.82, 2134.27) pg/mL and

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Table 4. Associations between FGF23 and carotid atherosclerosis

Independent variable: log ₁₀ FGF23		Crude	Model 1	Model 2	Model 3
Carotid plaque	β	0.45	1.07	1.34	1.48
	P	0.026	<0.001	0.012	0.011
	OR (95% CI)	1.57 (1.06, 2.34)	2.92 (1.72, 4.97)	3.74 (1.31, 10.55)	4.45 (1.42, 13.74)
CIMT	β (95% CI)	0.02 (0.01, 0.04)	0.04 (0.02, 0.06)	0.03 (0.01, 0.05)	0.02 (-0.01, 0.05)
	t	2.31	4.81	2.09	1.81
	P	0.022	<0.001	0.037	0.076
Carotid atherosclerosis	β	0.49	1.10	1.32	1.46
	P	0.018	<0.001	0.015	0.012
	OR (95% CI)	1.63 (1.09, 2.46)	3.01 (1.73, 5.26)	3.68 (1.25, 10.69)	4.39 (1.37, 14.01)

Model 1: adjusted for age and sex. Model 2: adjusted for Model 1 plus hypertension, diabetes, history of CVD, smoking, serum albumin, Ca, P, PTH, 25(OH)D, 1,25(OH)₂D, hsCRP, CKD stage, administration of lipid lowering drugs and vitamin D analogues. Model 3: adjusted for Model 2 plus TC, LDL-C, HDL-C, TG and BMI.

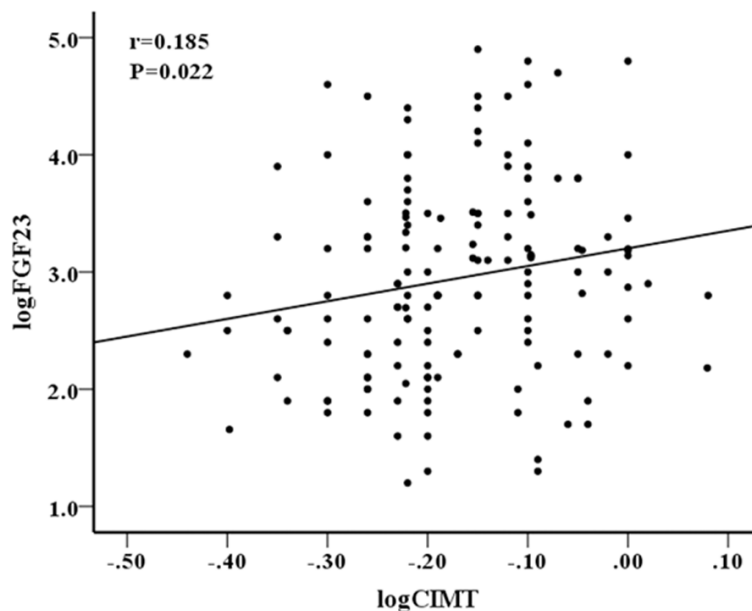


Figure 1. Serum FGF23 levels correlated positively with CIMT in patients with CKD stages 3-5D. Pearson's analysis showed that serum FGF23 levels were positively correlated with CIMT in patients with CKD stages 3-5D ($r=0.185$, $P=0.022$).

1736.10 (290.84, 10034.70) pg/mL, respectively. The FGF23 levels did not differ among the 4 groups ($P=0.11$). A per SD increase in log-FGF23 was associated with a higher risk of having carotid plaque in the crude model, model 1, and model 2. Further adjustment for components of the lipid profile and BMI did not attenuate the association (**Table 4**).

Pearson's analysis showed that serum FGF23 levels were positively correlated with CIMT ($r=0.185$, $P=0.022$) (**Figure 1**). We found statistically significant positive relationships between

FGF23 and CIMT in the crude model, model 1, and model 2. However, the association was lost after adjustment for components of the lipid profile and BMI (**Table 4**).

We found 19 (32%), 31 (62%), and 51 (73%) patients with carotid atherosclerosis in ND, PD and HD group, respectively. A per SD increase in log-FGF23 was associated with a higher risk of having carotid atherosclerosis in all the models (**Table 4**).

In addition, we determined the AUC for predicting carotid atherosclerosis from regression models (**Figure 2**). The AUC of model 1, which included baseline demographics only, was 0.834 (95% CI: 0.759, 0.909). A full model that included

serum FGF23 showed the highest model discrimination (model 3, AUC=0.940, 95% CI: 0.900, 0.980, $P<0.001$).

Discussion

Our study demonstrated that higher serum FGF23 levels were independently associated with lower TC, LDL-C, HDL-C levels, and BMI in the crude and multivariate analyses after adjusting for age, sex, traditional cardiovascular risk factors, albumin, markers of mineral metabolism, CKD stage, administration of lipid

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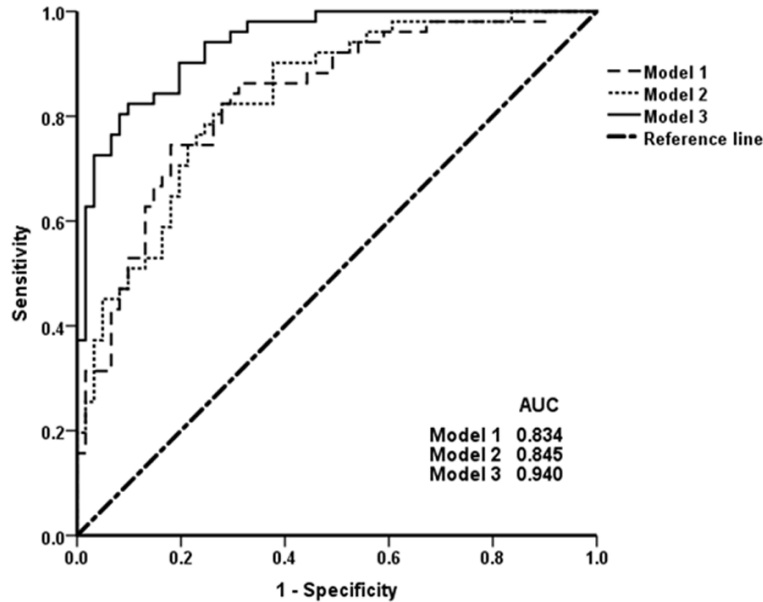


Figure 2. Receiver operating characteristic (ROC) curves for multivariate regression models to predict carotid atherosclerosis. Area under the curve (AUC) of ROC curve was compared. FGF23 had additive value in the prediction of carotid atherosclerosis in patients with CKD stages 3-5D ($P < 0.001$). Model 1: adjusted for age, sex, hypertension, diabetes, history of CVD, smoking, serum albumin, Ca, P, PTH, 25(OH)D, 1,25(OH)₂D, hsCRP, CKD stage, administration of lipid lowering drugs and vitamin D analogues; Model 2: model 1 plus TC, LDL-C, HDL-C, TG and BMI; Model 3: model 2 plus serum FGF23.

lowering drugs and vitamin D analogues in CKD stages 3-5D. We also determined that elevated FGF23 levels were related with a higher risk of carotid atherosclerosis.

To our knowledge, there has been limited research on the association of FGF23 levels with dyslipidemia in CKD population, and the participants were mostly confined to hemodialysis patients. Montford JR, *et al.* [13] evaluated 654 predominantly older black male patients on maintenance hemodialysis, and reported that higher plasma FGF23 levels were independently associated with lower HDL-C levels on unadjusted and multivariate analyses and with lower TC and LDL-C levels after adjusting for multiple confounders, but not with TG levels. In our research, the results for HDL-C and TG levels were similar, and we also discovered a stronger relationship between FGF23 and both TC and LDL-C levels in the unadjusted and multivariate analyses. The possible reasons for the inconsistent results could have been the different composition of race, age, and sex in the two cohorts, and the fact that we adjusted for more

confounders including smoking status, hsCRP levels, and the administration of vitamin D analogues. Active smoking is one of the important traditional cardiovascular risk factors that should be taken into consideration. CKD patients have a chronic microinflammatory state that may accelerate the atherogenesis process and lead to increased mortality [18]. Therefore, we incorporated into analysis the hsCRP levels, which are frequently used clinically to assess the microinflammatory state. Vitamin D has proven to be significantly associated with lipid metabolism [19]. The additional measurement of serum 25(OH)D and 1,25(OH)₂D levels together with information about the administration of vitamin D analogues made our results more powerful. A previous study reported by Ashikaga, *et al.* [14] was conducted among 196 hemodialysis pa-

tients. The authors discovered that FGF23 levels were inversely associated with TC and non-HDL-C, but not with LDL-C or HDL-C levels. In that study, the authors analyzed non-fasting blood samples for the laboratory tests, which is not the ideal method of lipid profile measurement. In addition, the authors were not able to adjust for several important confounding factors.

Previous studies have shown different findings with regard to the association between FGF23 level and BMI. Mirza, *et al.* [12] conducted an investigation in two independent community-based cohorts of individuals. FGF23 levels were found to be positively correlated with BMI and indicators of obesity. However, work by Montford JR, *et al.* and our study both noted contradictory observations that increased FGF23 levels were significantly associated with lower BMI. One explanation for the distinct results was that Mirza, *et al.* recruited participants from the community, who had significantly lower FGF23 levels. Moreover, it is worth mentioning that end-stage renal disease (ESRD)

patients have a markedly dissimilar body mass phenotype compared with age- and sex-matched controls in the general population [20]. Decreased BMI may indicate malnutrition, and has been shown to correlate with poor prognosis in advanced CKD patients [21, 22]. The underlying pathogenesis may be associated with microinflammation, oxidative stress, protein energy malnutrition (PEM), and atherosclerosis [23-25]. This hypothesis offers a reasonable explanation for the inverse relationship between FGF23 levels and BMI.

Since several bone-derived proteins, such as osteoprotegerin and osteocalcin, were reported to play a role in lipid metabolism [26, 27], it makes sense that FGF23 might have comparable biological effects. A study of *fgf23*-null mice model found that the absence of FGF23 induced aging-like features characterized not only by abnormal mineral homeostasis, but by cardiovascular calcification and disturbed lipid metabolism [28], suggesting that the effect of FGF23 is not confined solely to mineral regulation. The specific pathway and mechanism through which FGF23 might regulate lipid metabolism has not been elucidated. One hypothesis involves PTH and vitamin D as they both are regulated by FGF23 and are associated with fat mass and metabolic syndrome [29, 30]. Members of the FGF19 subfamily regulate diverse metabolic processes such as energy balance, carbohydrate, and lipid metabolism [11, 31]. FGF23 shares about 24% of the structural homology of FGF21 and 22% with FGF19 [32], which suggests possible molecular mimicry among the subfamily members. Laboratory research has demonstrated that FGF23 can signal through multiple FGFRs, including those belonging to other FGF19 subfamily members [33], among which FGFR4 plays an essential part in lipid and glucose metabolism [1].

Considering the close relationship among malnutrition, inflammation, and atherosclerosis (so-called MIA syndrome) in CKD, we further explored the association between FGF23 and carotid atherosclerosis. There are limited and contradictory data in this respect among CKD patients [14, 34-37]. FGF23 levels were found to have significantly positive correlation with CIMT and higher risk of carotid plaque and atherosclerosis in our study. Atherosclerosis contributes to increased cardiovascular risk and mortality in CKD patients, and dyslipidemia fre-

quently leads to accelerated atherosclerosis. It has been reported that elevated FGF23 levels were independently associated with poor cardiovascular outcomes and increased mortality in CKD patients [6-10]. However, the specific pathogenetic pathway is still largely obscure, and the current understanding is primarily related to the role of FGF23 in the regulation of mineral metabolism. We provide a hypothesis that the association between FGF23 and cardiovascular risk might, in part, be mediated through traditional risk factors beyond mineral disorder, that is, dyslipidemia.

Our study had several limitations. First, the cross-sectional nature of our study limited our ability to establish a causal relationship between FGF23 level and dyslipidemia. Second, the participants included non-dialysis, peritoneal dialysis, and hemodialysis CKD patients, which increased the variability of the patients' clinical characteristics. For these reasons, we adjusted for as many confounding factors as possible in the multivariate analyses in order to make the results more powerful. It should be noted that the cohort largely consisted of advanced stage CKD patients, thus narrowing the variability of different individuals. Third, we did not monitor or control the patients' dietary intake, so we cannot comment on the influence of diet.

In conclusion, we reported on an independent association between higher FGF23 levels and dyslipidemia, decreased BMI, an increased risk of carotid atherosclerosis in a cohort of patients with CKD stages 3-5D. Further research should be carried out to elucidate the specific mechanisms by which elevated FGF23 levels regulate lipid metabolism and lead to poor outcomes in CKD patients.

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Disclosure of conflict of interest

None.

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