

Low Plasma Levels of Fibroblast Growth Factor-21 in Patients with Peripheral Artery Disease

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Aim: Fibroblast growth factor-21 (FGF-21) is a metabolic regulator with beneficial effects on glucolipid metabolism. Since FGF-21 has lipid-lowering, anti-inflammatory and anti-oxidant properties, it may play a protective role against atherosclerosis. However, blood FGF-21 levels in coronary artery disease (CAD) or peripheral artery disease (PAD) have not been elucidated.

Methods: We measured plasma FGF-21 levels in 417 patients undergoing coronary angiography, who also had ankle-brachial index test for PAD screening.

Results: CAD was found in 224 patients (1-vessel [1-VD], $n=92$; 2-vessel [2-VD], $n=65$; 3-vessel disease [3-VD], $n=67$). No significant difference was found in the FGF-21 levels between 224 patients with CAD and 193 without CAD (median 26.0 vs. 25.9 pg/mL). FGF-21 levels in 4 groups of CAD(-), 1-VD, 2-VD, and 3-VD were 25.9, 37.2, 19.4, and 0.0 pg/mL. FGF-21 tended to be highest in 1-VD and lowest in 3-VD, but the difference did not reach statistical significance. PAD was found in 38 patients. Compared to the 379 patients without PAD, 38 with PAD had CAD more often (87% vs. 50%), especially 3-VD ($P<0.001$). FGF-21 levels were lower in patients with PAD than in those without PAD (0.0 vs. 30.7 pg/mL, $P<0.02$). In multivariate analysis, the FGF-21 level was an independent factor for PAD, but not for CAD. Odds ratio for PAD was 2.13 (95%CI=1.01-4.49) for a low FGF-21 level (<15.6 pg/mL).

Conclusion: No significant difference was found in the FGF-21 levels between patients with and without CAD. However, FGF-21 levels were low in patients with PAD, and were a factor for PAD independent of atherosclerotic risk factors.

Key words: Coronary artery disease, FGF-21, Peripheral artery disease

Introduction

Fibroblast growth factor (FGF)-21 has been recognized as a metabolic regulator with multiple beneficial effects on glucose and lipid metabolism and on insulin sensitivity^{1, 2}. FGF-21 is secreted from the liver, adipose tissues, and skeletal muscle². Transgenic mice overexpressing FGF-21 were shown to be resistant to diet-induced obesity¹, and the administration

of FGF-21 reduced blood glucose and triglyceride levels in animal models^{1, 3}. However, in humans, the blood levels of FGF-21 were reported to be high in patients with obesity, metabolic syndrome, and type 2 diabetes mellitus (DM)⁴⁻⁶. It is hypothesized that this paradoxical increase of FGF-21 may reflect a compensatory response^{4, 7}.

Recently, FGF-21 was suggested to play a protective role in atherosclerosis⁸. FGF-21 was demon-

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strated to have lipid-lowering, anti-inflammatory and anti-oxidant properties^{1, 8-11}). In apolipoprotein E-deficient mice, FGF-21 deficiency caused marked atherosclerotic plaque formation⁹), and the administration of FGF-21 ameliorated plaque formation¹²). With regard to the blood FGF-21 levels in patients with atherosclerotic diseases, Chow *et al.*¹³) investigated serum FGF-21 levels in 670 subjects in whom carotid intima-media thickness (IMT) was measured. They reported that FGF-21 levels positively correlated with carotid IMT in women but not in men. An *et al.*¹⁴) also measured serum FGF-21 levels in 213 subjects undergoing carotid ultrasound and reported that FGF-21 levels were higher in subjects with carotid plaques than in those without carotid plaques. Increased level of FGF-21 in patients with carotid atherosclerosis is thus considered to be a protective response to the development of atherosclerosis. However, blood FGF-21 levels in patients with coronary artery disease (CAD) remain controversial. Two studies^{15, 16}) reported that serum FGF-21 levels were higher in patients with CAD than in those without CAD, whereas another study¹⁷) reported that FGF-21 levels were not high in patients with CAD. Moreover, FGF-21 levels in patients with peripheral artery disease (PAD) have not been elucidated. Thus, our cross-sectional study was performed to elucidate the associations between plasma FGF-21 levels and CAD and PAD in 417 patients undergoing coronary angiography, who also had an ankle-brachial index (ABI) test to screen for PAD.

Methods

Study Patients

We investigated plasma FGF-21 levels in 417 consecutive patients undergoing elective coronary angiography for suspected CAD at Tokyo Medical Center, and who also had an ABI test to screen for PAD from July 2008 to April 2015. The ABI was measured in a supine position after 5 minutes of rest using the VaSera VS-1000 instrument (Fukuda Denshi, Tokyo), and PAD was defined as an ABI of < 0.9 ¹⁸). The severity of PAD was classified from stage I to stage IV according to the Fontaine's classification. Any of the patients with a history of percutaneous coronary intervention or cardiac surgery were excluded. Patients with acute myocardial infarction (AMI) were also excluded, because FGF-21 levels were reported to be elevated after AMI¹⁹). Hypertension was defined as blood pressures of $\geq 140/90$ mmHg or on drugs. Among the 417 patients, hypertension was present in 301 (72%) patients, and 253 (61%) patients were taking anti-hypertensive drugs. Hyper-

lipidemia was defined as a LDL-cholesterol level of > 140 mg/dl or on drugs. Hyperlipidemia was present in 206 (49%) patients, and 147 (35%) patients were taking statins. DM (fasting plasma glucose level of ≥ 126 mg/dl or on treatment) was present in 103 (25%) patients, and 76 (18%) patients were taking anti-diabetic drugs or were undergoing insulin treatment. Current smoking (≥ 1 cigarette/day) was found in 117 (28%) patients. Our study was approved by the ethics committee of our hospital (R07-054/R15-056) in June 2008. After written informed consent was obtained from the study patients, overnight-fasting blood samples were taken on the morning of the day when coronary angiography was performed.

Measurement of Plasma FGF-21 Levels

Blood samples were collected in EDTA-containing tubes. The plasma was stored at -80°C . Plasma FGF-21 levels were measured by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Human Fibroblast Growth Factor-21 ELISA Kit; Phoenix Pharmaceuticals, Inc., Belmont, USA) at Ochanomizu University, in accordance with the manufacturer's instructions. The minimum level detected by this assay was 15.6 pg/mL, and the level below the limit of detection (< 15.6 pg/mL) was described as 0.0 pg/mL. The range of values detected by this assay was 15.6 to 1000 pg/mL. The intra-assay and inter-assay coefficients of variation were $< 10\%$ and $< 15\%$, respectively.

Coronary Angiography

Coronary angiograms were recorded on a cineangiogram system (Philips Electronics Japan, Tokyo, Japan). CAD was defined as at least one coronary artery having $< 50\%$ luminal diameter stenosis. The severity of coronary atherosclerosis was represented as the number of $> 50\%$ stenotic vessels, the number of $> 50\%$ stenotic segments, and the severity score of stenosis. The degree of coronary artery stenosis in each segment was scored from 0 to 4 points (0, $\leq 25\%$; 1, 26-50%; 2, 51-75%; 3, 76-90%; 4, $< 90\%$ stenosis), and the severity score of stenosis was defined as the sum of the scores of all segments. Coronary artery segments were defined as 29 segments according to the Coronary Artery Surgery Study classification. All angiograms were evaluated by a single cardiologist (Y.M.), blinded to clinical and laboratory data.

Statistical Analysis

Differences between 2 groups were evaluated by the unpaired *t*-test for parametric variables, the Mann-Whitney *U* test for nonparametric variables, and the chi-squared test for categorical variables. Differences

Table 1. Clinical characteristics and plasma FGF-21 levels of patients with and without CAD

	CAD(-) (n = 193)	P value CAD(-) vs. CAD	CAD (n = 224)	1-VD (n = 92)	2-VD (n = 65)	3-VD (n = 67)	P value Among 4 groups
Age (years)	66 ± 11	< 0.01	70 ± 10	69 ± 10	69 ± 10	73 ± 7	< 0.01
Gender (male)	120 (62%)	< 0.01	171 (76%)	70 (76%)	46 (71%)	55 (82%)	0.02
BMI (kg/m ²)	23.6 ± 4.5	0.06	23.7 ± 3.3	23.9 ± 3.3	23.7 ± 3.2	23.3 ± 3.3	0.85
Hypertension	126 (65%)	< 0.01	175 (78%)	71 (77%)	49 (75%)	55 (82%)	0.03
SBP (mmHg)	131 ± 21	0.88	131 ± 23	129 ± 23	137 ± 21	128 ± 23	0.07
Diabetes mellitus	26 (13%)	< 0.01	77 (34%)	27 (29%)	24 (37%)	26 (39%)	< 0.01
Smoking	49 (25%)	0.20	68 (30%)	32 (35%)	17 (26%)	19 (28%)	0.50
Hyperlipidemia	74 (38%)	< 0.01	132 (59%)	52 (57%)	40 (62%)	40 (60%)	< 0.01
Statin	46 (24%)	< 0.01	101 (45%)	40 (43%)	28 (43%)	33 (49%)	< 0.01
LDL-C (mg/dL)	111 ± 29	0.18	115 ± 32	112 ± 33	118 ± 32	115 ± 30	0.33
HDL-C (mg/dL)	58 ± 15	< 0.01	51 ± 13	53 ± 14	49 ± 11	49 ± 12	< 0.01
FGF-21 level (pg/mL)	25.9 [0.0, 105.3]	0.38	26.0 [0.0, 86.1]	37.2 [0.0, 88.6]	19.4 [0.0, 77.6]	0.0 [0.0, 63.4]	0.21
FGF-21 < 15.6 pg/mL	81 (42%)	0.50	104 (46%)	36 (39%)	32 (49%)	36 (54%)	0.30
PAD (ABI < 0.9)	5 (3%)	< 0.01	33 (15%)	9 (10%)	6 (9%)	18 (27%)	< 0.01
Antiplatelet drugs	62 (32%)	< 0.01	134 (60%)	50 (54%)	46 (71%)	38 (57%)	< 0.01

Data represent the mean ± SD or the number (%) of patients, with the exception of FGF-21 level which is presented as the median value and interquartile range. BMI indicates body mass index; SBP, systolic blood pressure; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol.

among ≥ 3 groups were evaluated by ANOVA with Scheffe's test for parametric variables, by Kruskal-Wallis test for nonparametric variables, and by chi-squared test for categorical variables. Correlations between FGF-21 levels and the severity of coronary atherosclerosis were evaluated using Spearman's rank correlation test. A multiple logistic regression analysis was used to determine independent factors associated with PAD or CAD, including FGF-21 and conventional risk factors. A *P* value of < 0.05 was considered statistically significant. Results are presented as the mean ± SD or the median value.

Results

Among the 417 study patients, CAD was found in 224 patients (1-vessel disease [1-VD], *n*=92; 2-vessel disease [2-VD], *n*=65; and 3-vessel disease [3-VD], *n*=67). In comparison to the 193 patients without CAD, 224 patients with CAD were older and had a male predominance and higher prevalence of hypertension, DM and hyperlipidemia (Table 1). However, no significant difference was found in plasma FGF-21 levels between patients with and without CAD (median 26.0 pg/mL vs. 25.9 pg/mL, *P*=0.38). FGF-21 levels in the 4 groups of CAD(-), 1-VD, 2-VD, and 3-VD were median 25.9, 37.2, 19.4, and 0.0 pg/mL, respectively. Among these 4 groups, FGF-21 levels tended to be highest in patients with 1-VD and lowest in those with 3-VD; however, these differences

did not reach statistical significance (Fig. 1). Spearman's rank correlation test revealed no significant correlations between FGF-21 levels and the number of > 50% stenotic coronary segments (*r*=0.08, *P*=0.11) or the severity score of stenosis (*r*=0.09, *P*=0.08).

The 417 study patients were divided into 2 groups according to the presence or absence of DM. However, between 103 patients with DM and 314 without DM, no significant difference was found in FGF-21 levels (median 27.4 pg/mL vs. 25.6 pg/mL, *P*=0.82). Among the 314 patients without DM, FGF-21 levels did not differ between patients with and those without CAD (median 18.5 pg/mL vs. 29.6 pg/mL, *P*=0.25). FGF-21 levels tended to be highest in patients with 1-VD and lowest in those with 3-VD; however, there were no significant differences among CAD(-), 1-VD, 2-VD, and 3-VD groups (median 29.6, 36.3, 0.0, and 0.0 pg/mL, respectively). Moreover, among the 103 patients with DM, FGF-21 levels did not differ between patients with and without CAD (median 29.8 pg/mL vs. 18.8 pg/mL, *P*=0.96) or among CAD(-), 1-VD, 2-VD and 3-VD groups (median 18.8, 38.2, 31.3, and 6.7 pg/mL).

Among the 417 study patients, PAD (ABI < 0.9) was found in 38 (9%) patients (5 out of the 193 [3%] patients without CAD and 33 out of the 224 [15%] with CAD). Compared with 379 patients without PAD, 38 patients with PAD were older and had a higher prevalence of hypertension and smoking (Table 2). Notably, FGF-21 levels in patients with PAD were

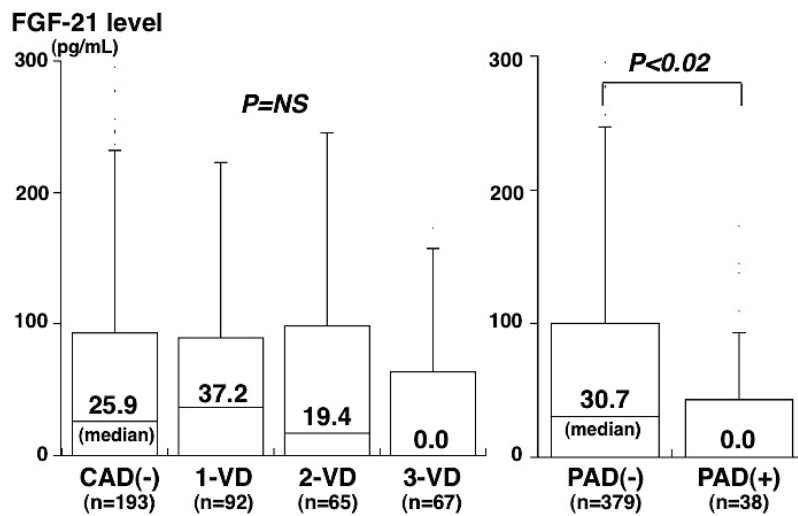


Fig. 1. Plasma FGF-21 levels and the number of stenotic coronary vessels or the presence of PAD

FGF-21 levels tended to be highest in 1-VD and lowest in 3-VD, but there was no significant difference among the 4 groups ($P=NS$) (left). However, FGF-21 levels in PAD were significantly lower than those in PAD(-) ($P<0.02$) (right). The central line represents the median, and the boxes span from the 25th to 75th percentiles. 1-VD, 1-vessel disease; 2-VD, 2-vessel disease; 3-VD, 3-vessel disease; PAD, peripheral artery disease.

significantly lower than those in patients without PAD (median 0.0 pg/mL vs. 30.7 pg/mL, $P=0.02$) (Fig. 1). FGF-21 levels in patients with PAD were more often below the limit of detection (<15.6 pg/mL) than in those without PAD (63% vs. 43%, $P=0.03$) (Table 2). Of the 38 patients with PAD, 22 and 16 patients were found to have stage I and II PAD, respectively. FGF-21 levels in patients with stage II PAD tended to be lower (median 0.0 pg/mL vs. 6.4 pg/mL) and to be more often <15.6 pg/mL (69% vs. 59%) than in those with stage I PAD. However, these differences did not reach statistical significance. Notably, patients with PAD more often had CAD (87% vs. 50%), especially 3-VD (47% vs. 13%), than those without PAD ($P<0.01$) (Table 2). FGF-21 levels in PAD patients with CAD(-), 1-VD, 2-VD, and 3-VD were median 12.8, 18.5, 0.0, and 0.0 pg/mL, respectively.

Between patients with and without PAD, there was no significant difference in the prevalence of DM (37% vs. 23%). Among the 314 patients without DM, FGF-21 levels tended to be lower in patients with PAD than in those without PAD (median 0.0 pg/mL vs. 29.4 pg/mL). Among the 103 patients with DM, FGF-21 levels also tended to be lower in patients with PAD than in those without PAD (median 0.0 pg/mL vs. 31.3 pg/mL).

To elucidate the independent associations between FGF-21 levels and CAD or PAD, variables (age, gender, hypertension, hyperlipidemia, statin use,

DM, smoking, HDL-cholesterol, and FGF-21 levels) were entered into a multiple logistic regression model. The FGF-21 level was found to be a significant factor for PAD independent of atherosclerotic risk factors, but not for CAD or 3-VD (Table 3) (model 1). The odds ratio for PAD in patients with a low FGF-21 level (<15.6 pg/mL) was 2.13 (95% CI = 1.01-4.49, $P<0.05$). In the model including CAD as well as atherosclerotic risk factors and FGF-21 levels, FGF-21 level and CAD were independent factors associated with PAD (model 2). However, in the model including 3-VD as well as atherosclerotic risk factors and FGF-21 levels, 3-VD was a significant factor associated with PAD (model 3).

Discussion

In the present study, there was no significant difference in plasma FGF-21 levels between patients with and without CAD. No significant correlation was found between FGF-21 levels and the severity of coronary atherosclerosis, defined as the numbers of stenotic vessels and segments. However, the present study showed that FGF-21 levels were lower in patients with PAD than in those without PAD and that they were a significant factor associated with PAD independent of atherosclerotic risk factors.

FGF-21 is recognized as a metabolic regulator with beneficial effects on glucolipid metabolism and

Table 2. Clinical characteristics and plasma FGF-21 levels of patients with and without PAD

	PAD(-) (n = 379)	PAD (n = 38)	P value
Age (years)	67 ± 10	75 ± 6	< 0.01
Gender (male)	263 (69%)	28 (74%)	0.80
BMI (kg/m ²)	23.7 ± 4.0	22.7 ± 3.2	0.12
Hypertension	267 (70%)	34 (89%)	0.03
Systolic blood pressure (mmHg)	131 ± 20	131 ± 30	0.92
Diabetes mellitus	89 (23%)	14 (37%)	0.70
Smoking	103 (27%)	14 (37%)	0.30
Hyperlipidemia	182 (48%)	24 (63%)	0.70
Statin	125 (33%)	22 (58%)	< 0.01
LDL-cholesterol (mg/dL)	113 ± 31	113 ± 29	0.92
HDL-cholesterol (mg/dL)	55 ± 14	49 ± 11	0.01
FGF-21 level (pg/mL)	30.7 [0.0, 100.5]	0.0 [0.0, 39.4]	0.02
FGF-21 < 15.6 pg/mL	163 (43%)	24 (63%)	0.03
CAD	191 (50%)	33 (87%)	< 0.01
1-VD	83 (22%)	9 (24%)	0.95
2-VD	59 (16%)	6 (6%)	0.90
3-VD	49 (13%)	18 (47%)	< 0.01
Antiplatelet drugs	171 (45%)	25 (66%)	0.03

Data represent the mean ± SD or the number (%) of patients, with the exception of FGF-21 level which is presented as the median value and interquartile range.

insulin sensitivity^{1, 2}). Blood FGF-21 levels were reported to be high in patients with obesity, metabolic syndrome and DM⁴⁻⁶). This paradoxical increase in FGF-21 levels has been suggested to be a compensatory response^{4, 7}). Because FGF-21 has lipid-lowering, anti-inflammatory and anti-oxidant properties^{1, 8-11}), FGF-21 is considered to play a protective role against atherosclerosis⁸). In apoE-deficient mice, FGF-21 deficiency caused marked atherosclerosis⁹), whereas FGF-21 administration ameliorated atherosclerosis¹²). However, blood FGF-21 levels in CAD patients remain controversial. Lin *et al.*¹⁵) reported that serum FGF-21 levels in 135 patients with CAD were higher than those in 61 controls; however, the study included patients with AMI. In patients with AMI, FGF-21 levels are shown to be elevated and to remain high for 7 days after AMI¹⁹). Kim *et al.*²⁰) measured serum FGF-21 levels in 120 patients undergoing coronary angiography (30 CAD[-] DM[-], 30 CAD[+] DM[-], 30 CAD[-] DM[+] and 30 CAD[+] DM[+] patients). They excluded AMI patients and reported that FGF-21 levels were higher in patients with CAD than in those without CAD, but only among DM(-) patients. In contrast, Lee *et al.*¹⁷) investigated serum FGF-21 levels in 189 patients undergoing coronary computed tomography. They showed that FGF-21 levels in 60 patients with CAD were not higher than those in 129

patients without CAD. Our study investigated plasma FGF-21 levels in 417 patients undergoing elective coronary angiography. AMI patients were excluded. However, FGF-21 levels in 224 patients with CAD were not higher than those in 193 patients without CAD and were not a significant factor for CAD, regardless of the presence or absence of DM.

Recently, Li *et al.*²¹) investigated the association between FGF-21 levels and mortality in 1668 patients with CAD. They showed that both the highest and lowest FGF-21 levels were associated with an increased risk of cardiovascular mortality, suggesting a U-shaped association between FGF-21 levels and mortality. Moreover, Jung *et al.*²²) recently reported the U-shaped association between FGF-21 levels and diabetic retinopathy in 227 patients with DM. Both highest and lowest FGF-21 levels were associated with diabetic retinopathy, but not with nephropathy or neuropathy. They suggested that a very low FGF-21 level itself may be associated with diabetic retinopathy and also a relatively elevated FGF-21 level may be a compensatory increase to protect against microvascular complication. In our study, FGF-21 levels in CAD(-), 1-VD, 2-VD, and 3-VD groups were 25.9, 37.2, 19.4, and 0.0 pg/mL, respectively, and tended to be highest in patients with 1-VD and lowest in those with 3-VD, suggesting an inverted U-shaped association. Because

Table 3. Factors associated with PAD and CAD

	Model 1		Model 2		Model 3	
	OR (95%CI)	P value	OR (95%CI)	P value	OR (95%CI)	P value
PAD						
Age (per 10 yrs)	2.33 (1.44-3.77)	< 0.01	2.27 (1.39-3.70)	< 0.01	2.33 (1.40-3.87)	< 0.01
Gender (male)	---	NS	---	NS	---	NS
Hypertension	---	NS	---	NS	---	NS
Hyperlipidemia	---	NS	---	NS	---	NS
Statin	2.27 (1.10-4.66)	0.03	---	NS	---	NS
HDL-C (per 1 mg/dL)	0.96 (0.93-0.99)	0.02	---	NS	---	NS
Smoking	---	NS	---	NS	---	NS
Diabetes mellitus	---	NS	---	NS	---	NS
Anti-platelet drugs	---	NS	---	NS	---	NS
FGF-21(< 15.6 pg/mL)	2.10 (1.01-4.40)	0.04	2.15 (1.02-4.49)	0.04	---	NS
CAD			5.23 (1.96-13.9)	< 0.01		
3-vessel disease					4.86 (2.34-10.1)	< 0.01
CAD						
Age (per 10 yrs)	1.50 (1.20-1.88)	< 0.01	1.43 (1.14-1.79)	< 0.01		
Gender (male)	2.22 (1.30-3.79)	< 0.01	2.21 (1.29-3.80)	< 0.01		
Hypertension	---	NS	---	NS		
Hyperlipidemia	2.53 (1.57-4.09)	< 0.01	2.48 (1.53-4.01)	< 0.01		
Statin	---	NS	---	NS		
HDL-C (per 1 mg/dL)	0.97 (0.95-0.99)	< 0.01	0.97 (0.95-0.99)	< 0.01		
Smoking	---	NS	---	NS		
Diabetes mellitus	2.14 (1.23-3.69)	< 0.01	2.13 (1.23-3.69)	< 0.01		
Anti-platelet drugs	2.58 (1.64-4.05)	< 0.01	2.51 (1.59-3.96)	< 0.01		
FGF-21(< 15.6 pg/mL)	---	NS	---	NS		
PAD			3.37 (1.20-9.45)	0.02		
3-vessel disease						
Age (per 10 yrs)	1.93 (1.39-2.70)	< 0.01	1.82 (1.29-2.56)	< 0.01		
Gender (male)	2.54 (1.21-5.31)	0.01	2.23 (1.07-4.64)	0.03		
Hypertension	---	NS	---	NS		
Hyperlipidemia	---	NS	---	NS		
Statin	1.94 (1.09-3.45)	0.02	---	NS		
HDL-C (per 1 mg/dL)	0.97 (0.95-0.99)	0.02	0.97 (0.95-0.99)	0.02		
Smoking	---	NS	---	NS		
Diabetes mellitus	---	NS	---	NS		
Anti-platelet drugs	---	NS	---	NS		
FGF-21(< 15.6 pg/mL)	---	NS	---	NS		
PAD			4.14 (1.96-8.74)	< 0.01		

OR indicates odds ratio; 95%CI, 95% confidence interval.

FGF-21 levels were shown to be high in subjects with carotid plaques¹⁴, patients with mild CAD, such as 1-VD, may have high FGF-21 levels, which reflect a protective response to CAD progression. In contrast, patients with severe CAD, such as 3-VD, may have low FGF-21 levels, thereby leading to the development of severe CAD. However, to elucidate the role of FGF-21 in CAD progression, further studies are needed to show the association between FGF-21 levels

and the progression of CAD in a prospective manner.

Atherosclerosis is a progressive disease that affects multiple vascular beds, and patients with CAD are known to be often complicated by PAD^{18, 23}. In our study, PAD was found in 5 of the 193 patients without CAD (3%) and 33 of the 224 with CAD (15%). This prevalence of PAD was similar to that reported by Lee *et al.*¹⁸) (5 of 119 without CAD (4%) and 385 of 2424 with CAD (16%) among 2543 patients

undergoing coronary angiography). Regarding blood FGF-21 levels in patients with PAD, only one study investigated the association between FGF-21 levels and PAD in 504 Chinese patients with type 2 DM²⁴. They reported that FGF-21 levels were higher in female patients with PAD than in those without PAD, but FGF-21 levels tended to be lower in male patients with PAD than in those without PAD. Because patients with PAD were reported to have skeletal muscle mitochondrial dysfunction²⁵ and because mitochondrial dysfunction enhances FGF-21 expression²⁶, patients with PAD may have an increased FGF-21 expression associated with muscle mitochondrial dysfunction. However, our study demonstrated that plasma FGF-21 levels were significantly lower in patients with PAD than in those without PAD, and that FGF-21 levels in patients with PAD were often very low (below the limit of detection). The low FGF-21 level was found to be a significant factor for PAD, independent of atherosclerotic risk factors. Notably, patients with PAD often had CAD, especially 3-VD. In multivariate analysis, 3-VD was significantly associated with PAD. FGF-21 levels in PAD patients with CAD(-), 1-VD, 2-VD and 3-VD were 12.8, 18.5, 0.0 and 0.0 pg/mL, respectively. Our findings suggest that patients with PAD, especially those with 3-VD, have very low FGF-21 levels, which thereby lead to the development of severe atherosclerosis.

Our study has several limitations. First, angiography was used to evaluate coronary atherosclerosis. Angiography cannot visualize plaques and only shows lumen characteristics. Second, PAD was present in 5 out of 193 patients without CAD (3%) and in 33 out of 224 with CAD (15%). Although atherosclerotic plaques in peripheral arteries were reported to be common in patients without any symptoms of PAD, especially in those with CAD²⁷, this prevalence of PAD was similar to that reported by Lee *et al.*¹⁸ (4% of patients without CAD and 16% of those with CAD). However, the small number of patients with PAD ($n=38$) was one of the major limitations. Moreover, in our study, an ABI test was used to screen for PAD, and PAD was defined as an ABI of <0.9 ¹⁸. Angiography or computed tomography were not always performed to confirm the diagnosis of PAD. This is another of the study limitations. Third, our study was cross-sectional in nature, and it could not establish causality, since it only showed some associations and proposed some hypotheses. Finally, all four of the previous studies on FGF-21 levels in CAD patients were reported from China and Korea (2 studies from each country)^{15-17, 20}. Since our study was performed in Japanese patients, the results cannot be applicable to other ethnic populations.

In conclusion, plasma FGF-21 levels were not different between patients with and without CAD and were not a significant factor for CAD. However, FGF-21 levels were found to be low in patients with PAD, especially in PAD patients with 3-VD, and to be a significant factor associated with PAD independent of atherosclerotic risk factors. Our results suggest that FGF-21 may play a protective role against the development of PAD.

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Conflict of Interest

Our study has no conflict of interest to disclose.

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