

ORIGINAL**Correlation of fibroblast growth factor 21 serum levels with metabolic parameters in Japanese subjects**

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Abstract : Objects : Since serum level of fibroblast growth factor 21 (FGF21) has been implicated as a potential biomarker for the early detection of the metabolic syndrome and type 2 diabetes, we examined how FGF21 serum levels are correlated with metabolic parameters in Japanese subjects. **Methods :** FGF21 levels were analyzed by enzyme-linked immunosorbent assays. Spearman's correlation and multiple stepwise regression analyses were used to examine the relationship between serum FGF21 and other factors. A Mann-Whitney *U* test was performed between the normal and high groups for triglycerides and systolic blood pressure (BP) respectively. **Results :** By univariate correlation analysis, serum FGF21 levels were significantly associated with triglyceride levels, systolic BP, diastolic BP, pulse pressure, body mass index (BMI), age, fasting plasma glucose (FPG) levels, and total cholesterol levels. Multiple regression analysis (adjusted for age, gender, and BMI) showed that serum FGF21 levels were independently and significantly associated with triglyceride levels and systolic BP. Serum FGF21 levels were significantly higher in subjects with high triglyceride levels and high systolic BP compared with those who had normal triglyceride levels and normal systolic BP respectively. **Conclusions :** This study found that FGF21 levels might be a biomarker for some metabolic disorders associated with metabolic syndrome. *J. Med. Invest.* 61 : 28-34, February, 2014

Keywords : FGF21, metabolic syndrome, triglycerides, systolic BP, biomarker

INTRODUCTION

Fibroblast growth factor 21 (FGF21) is a member of the FGF subfamily and was originally isolated from the liver (1). FGF21 is a member of the diverse

“hormone-like” subgroup within the FGF subfamily that also includes FGF19 and FGF23 (2). Hormone-like FGF signaling disorders caused by hereditary diseases or tumors are associated with metabolic disease (3). In addition, serum hormone-like

Abbreviations : FGF21, fibroblast growth factor 21 ; BP, blood pressure ; BMI, body mass index ; FPG, fasting plasma glucose ; NAFLD, nonalcoholic fatty liver disease ; HDL, high-density lipoprotein ; HbA1c, hemoglobin A1c ; LDL, low-density lipoprotein ; S.D., standard deviation.

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FGF levels are significantly affected in some metabolic disorders (3). Serum FGF19 levels are significantly increased in patients on chronic hemodialysis (4) and significantly decreased in non-alcoholic fatty liver disease (NAFLD) patients (5). Serum FGF23 levels are also greatly increased in patients with renal failure (6, 7). Numerous recent studies have focused on FGF21 as a key regulator or biomarker. Li *et al.* reported that FGF21 levels are increased in NAFLD patients and suggested that serum FGF21 could be potentially used as a biomarker for NAFLD (8). Serum FGF21 levels are also increased in patients with type 2 diabetes, obesity (9-11) and Cushing's syndrome (12). In contrast, serum FGF21 levels are decreased in patients with anorexia nervosa (13) and in patients with type 1 diabetes (14). Eto *et al.* reported that the serum concentration of FGF21 is associated with clinical parameters in Japanese patients with type 2 diabetes (15). In addition, FGF21 has been shown to be an independent predictor of type 2 diabetes (16). Taken together, these results suggest that FGF21 could be a biomarker for metabolic disorders such as type 2 diabetes. Further understanding of the functions of hormone-like FGFs will provide clues to their roles in metabolism and clinical treatments for metabolic diseases (3).

According to the International Diabetes Federation (17) metabolic syndrome is defined as central obesity and any two of the following: (1) high triglycerides defined as ≥ 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality; (2) reduced high-density lipoprotein (HDL) cholesterol defined as < 40 mg/dL (1.03 mmol/L) in men or < 50 mg/dL (1.29 mmol/L) in women, or specific treatment for this lipid abnormality; (3) high BP of systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg, or treatment of previously diagnosed hypertension; (4) high FPG defined as ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes (17). This indicates that metabolic syndrome is strongly associated with obesity, triglycerides, HDL cholesterol, BP, and FPG.

Tokushima Prefecture, Japan, has a high rate of obesity: 37% of men are obese (18, 19). To prevent an onset of metabolic syndrome in Tokushima, suitable biomarkers must be found that are associated with the clinical parameters affected in metabolic syndrome, since these biomarkers might predict an onset of metabolic syndrome. However, no studies to date have reported a correlation between FGF21 levels and clinical parameters associated with

metabolic syndrome in Japan, including Tokushima. This study is a first report concerning serum FGF21 levels and metabolic disorders in Japanese subjects. In this study, we measured the serum concentration of FGF21 in 998 Japanese subjects in Tokushima Prefecture and investigated its relationship with clinical parameters. We found that serum FGF21 levels correlated with some clinical parameters used in diagnosing metabolic syndrome. In addition, we showed that people with either high triglycerides or systolic BP exhibit higher levels of serum FGF21 than people with normal triglycerides levels or systolic BP.

MATERIALS AND METHODS

Recruitment and eligibility

A total of 1068 participants aged 20 to 65 years old participated in a cross-sectional study conducted by the Clinical Research Center for Diabetes at the Tokushima University Hospital. This study was approved by the Ethics Committees of the Tokushima University Hospital. Further inclusion criteria were the absence of any other disorder or medication known to affect the serum FGF21 level. All patients gave informed consent to participate in the study. To evaluate clinical parameters accurately, 70 participants were excluded because they were either receiving medication for metabolic disorders such as diabetes, dyslipidemia and hypertension, or were not fasting at the time of blood examination. The remaining 998 subjects (752 men and 246 women) were analyzed in this study.

Anthropometric and biochemical measurements

Height (cm) and weight (kg) were measured. BMI was calculated as weight in kilograms divided by the square of height in meters. Systolic BP and diastolic BP were measured by standard methods. Blood samples were drawn after overnight fasting by venipuncture to measure levels of serum glucose, hemoglobin A1c (HbA1c), triglyceride, total cholesterol, HDL cholesterol, and low-density lipoprotein (LDL) cholesterol. These factors were measured by standard enzymatic methods. FGF21 levels were analyzed by enzyme-linked immunosorbent assays (BioVendor, Modrice, Czech Republic) according to the manufacturer's instructions. Intra- and inter-assay precision of ELISA were 3.4% and 6.1% respectively. HbA1c levels were analyzed by a high-performance liquid chromatography assay and

expressed by NGSP values.

Definition of normal and high groups

Subjects were divided into two groups by either triglyceride levels or systolic BP. Subjects with triglyceride levels lower than 150 mg/dL were in the normal group (18~149 mg/dl, n=814). Those with triglyceride levels equal to or higher than 150 mg/dl were in the high group (150~2819 mg/dl, n=184). Subjects with systolic BP lower than 130 mmHg were in the normal group (82~129 mmHg, n=798), and those with systolic BP equal to or higher than 130 mmHg were included in the high group (130~202 mmHg, n=200) (20).

Statistical analysis

Statistical analyses were performed using SPSS 19.0 software (SPSS ; Chicago, IL, USA). Because serum FGF21 levels were not normally distributed, they were logarithmically converted and used for correlation analysis and multiple stepwise regression analysis. Spearman's correlation and multiple stepwise regression analyses were used to examine the relationship between serum FGF21 and other factors. Factors that were significantly correlated to serum FGF21 were selected for multiple stepwise regression analysis. A Mann-Whitney *U* test was performed between the normal and high groups for triglycerides and systolic BP, respectively. All data were presented as mean \pm standard deviation (S.D). In all statistical analyses, a *p* value less than 0.05 was considered statistically significant.

RESULTS

In this study, 998 subjects were analyzed (men/women=752/246). Clinical parameters of study subjects are in Table 1. The distribution of serum FGF21 concentrations was broad (16-4577 pg/ml, median value 189 pg/ml), and the mean \pm S.D value was 272 \pm 388 pg/ml. No significant difference was seen in serum FGF21 levels between men and women (280 \pm 405 pg/ml vs. 248 \pm 329 pg/ml, *p*=0.917).

Using univariate correlation analysis, serum FGF21 levels were most significantly associated with triglyceride levels ($r=0.200$, $p<0.001$, Figure 1). The second most significant association was observed between serum FGF21 and systolic BP ($r=0.167$, $p<0.001$, Figure 2). Serum FGF21 levels were also associated with diastolic BP ($r=0.145$, $p<0.001$), BMI

($r=0.114$, $p<0.001$), age ($r=0.105$, $p=0.001$), FPG ($r=0.093$, $p=0.003$), pulse pressure ($r=0.086$, $p=0.006$) and total cholesterol ($r=0.066$, $p=0.036$) levels, although correlation coefficients were low (Table 2). No significant correlation was seen between FGF21 levels and gender, HbA1c, LDL cholesterol, or HDL cholesterol levels (Table 2).

We conducted multiple regression analysis adjusting for age, gender, and BMI. Serum FGF21 levels were independently and significantly associated with triglyceride levels and systolic BP (Table 3).

Table 1. Clinical parameters of study subjects

Parameter	Value
n	998
Age (years)	41 \pm 10
BMI (kg/m ²)	23.4 \pm 3.4
Systolic BP (mmHg)	120 \pm 15
Diastolic BP (mmHg)	75 \pm 11
Pulse pressure (mmHg)	45 \pm 8.3
Triglyceride (mg/dl)	107 \pm 76
Total cholesterol (mg/dl)	198 \pm 32
LDL cholesterol (mg/dl)	120 \pm 30
HDL cholesterol (mg/dl)	57 \pm 15
FPG (mg/dl)	91 \pm 9
HbA1c (%)	5.13 \pm 0.34
FGF21 (pg/ml)	272 \pm 388

Data are expressed as mean \pm S.D.

Abbreviations : BMI, body mass index ; BP, blood pressure ; LDL, low-density lipoprotein ; HDL, high-density lipoprotein ; FPG, fasting plasma glucose ; HbA1c, hemoglobin A1c ; FGF21, fibroblast growth factor 21.

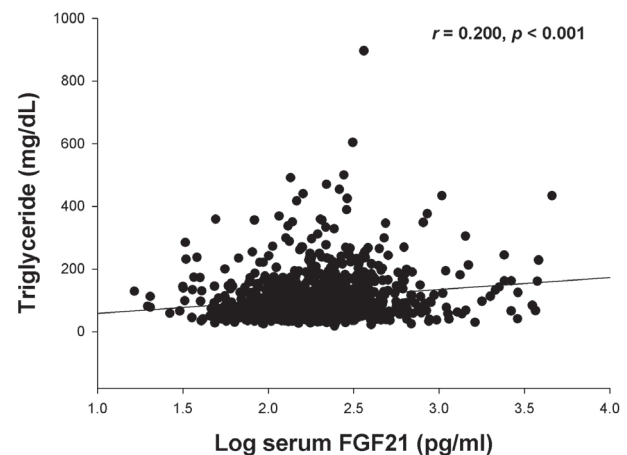


Figure 1. Correlation between log serum FGF21 levels and triglyceride levels. Log serum FGF21 concentrations were plotted against triglyceride concentrations. Line indicates the calculated linear correlation curve.

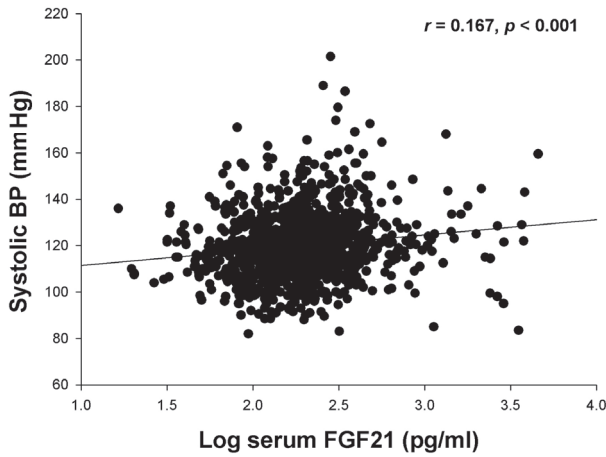


Figure 2. Correlation between log serum FGF21 levels and systolic BP. Log serum FGF21 concentrations were plotted against systolic BP. Line indicates the calculated linear correlation curve.

Table 2. Factors associated with serum FGF21 levels

Factor	FGF21 (n=998)	
	r	p
Age	0.105	0.001
Gender	-0.034	0.285
BMI	0.114	< 0.001
Systolic BP	0.167	< 0.001
Diastolic BP	0.145	< 0.001
Pulse pressure	0.086	0.006
Triglyceride	0.200	< 0.001
Total cholesterol	0.066	0.036
LDL cholesterol	0.046	0.150
HDL cholesterol	-0.052	0.100
FPG	0.093	0.003
HbA1c	0.05	0.116

Spearman’s correlation analysis was used to examine an association between serum FGF21 and other factors. *r* is a correlation coefficient and a *p* value less than 0.05 was considered statistically significant.

Abbreviations : BMI, body mass index ; BP, blood pressure ; LDL, low-density lipoprotein ; HDL, high-density lipoprotein ; FPG, fasting plasma glucose ; HbA1c, hemoglobin A1c.

Table 3. Multiple stepwise regression analysis adjusted for age, gender, and BMI

Factor	FGF21 (n=998)	
	β	<i>p</i>
Triglyceride	0.140	< 0.001
Systolic BP	0.112	0.001

Factors significantly associated with serum FGF21 were selected for multiple stepwise regression analysis. β is a standardized coefficient and a *p* value less than 0.05 was considered statistically significant.

Abbreviations : BMI, body mass index ; BP, blood pressure.

Finally, we performed Mann-Whitney *U* tests to determine if serum FGF21 was elevated in people with higher levels of either triglycerides or systolic BP. As shown in Figures 3 and 4, the serum levels of FGF21 were significantly higher in subjects with high triglyceride levels or high systolic BP than in those with triglyceride or systolic BP values in the normal range.

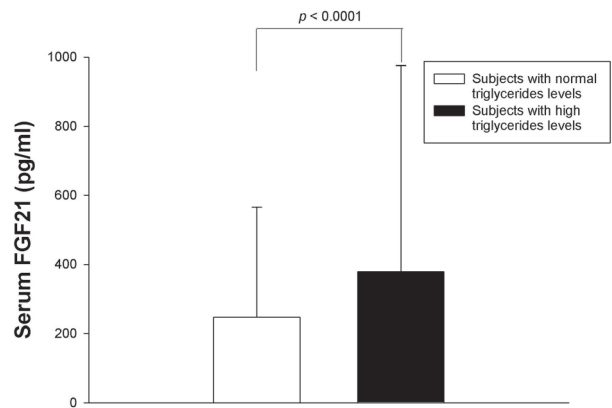


Figure 3. Serum FGF21 concentration in subjects with normal triglycerides levels (n=814) and subjects with high triglycerides levels (n=184).

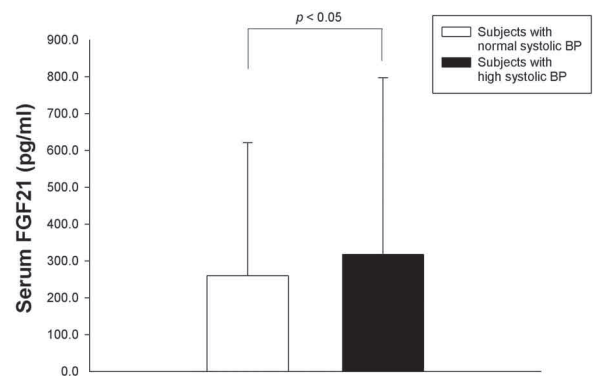


Figure 4. Serum FGF21 concentration in subjects with normal systolic BP (n=798) and subjects with high systolic BP (n=200).

DISCUSSION

In this study we analyzed clinical parameters associated with serum FGF21 levels in subjects recruited in Tokushima, Japan, who were not under medication for metabolic disorders. Our results showed that serum FGF21 levels were independently and significantly associated with triglyceride levels and systolic BP. These results were consistent with a previous study of type 2 diabetes patients

(15), which verifies the reliability and validity of our study design and data. We compared for the first time serum levels of FGF21 between two groups : those with either high triglyceride levels or high systolic BP and those with either of these parameters in the normal range, by applying cut-off values used to diagnose metabolic syndrome (17).

As shown in Figures 3 and 4, serum FGF21 levels were significantly elevated in people with higher triglyceride levels or higher systolic BP. Because these factors are associated with metabolic syndrome, these results suggested that serum FGF21 level could be a promising biomarker for metabolic disorders associated with metabolic syndrome. A prospective cohort study will be necessary to establish the significance of FGF21 as a biomarker for metabolic disorders associated with metabolic syndrome and metabolic syndrome itself.

The serum level of FGF21 was independently and significantly associated with systolic BP (Table 2). Furthermore, subjects with high systolic BP exhibited a significantly higher level of FGF21 (Figure 4). Hypertension has been known to increase the risk of cardiovascular disease (21). High levels of triglycerides in the bloodstream, which exhibited an association with high FGF21 level (Figure 1 and 3), has been also linked to atherosclerosis (22), which, in turn, increases the risk of heart disease and stroke. In fact, Lin and colleagues have reported that increased serum FGF21 level is associated with coronary heart disease, which is independent of adverse lipid profile (23). In addition, our data revealed that FGF21 levels were significantly associated with pulse pressure that has been known to be a cardiovascular risk factor independent of systolic blood pressure (24). Hence, FGF21 might serve as a biomarker for the risk of cardiovascular diseases. Further research is necessary to test this possibility.

Previous reports demonstrated that serum triglyceride levels and hepatic triglyceride contents are reduced by FGF21 administration (25, 26). Thus, as suggested previously (10), the positive association between FGF21 levels and triglyceride levels found in this study might indicate a state of “FGF21 resistance”, in which FGF21 fails to maintain triglyceride levels within the normal range. Triglycerides, as one of the major components of very low-density lipoprotein and chylomicrons, are important in lipid metabolism as an energy source and transporters for dietary fat. We found that FGF21 levels were significantly increased in subjects with triglyceride

levels equal to or higher than 150 mg/dl (Figure 3). Thus, our results suggest that serum FGF21 level could be a biomarker for abnormal lipid metabolism by reporting the degree of FGF21 resistance.

Fibrates, antihyperlipidemic agents and agonists for the transcription factor peroxisome proliferator-activated receptor (PPAR)- α , are known to upregulate the expression and serum concentration of FGF21 (27). Furthermore, Dutchak and colleagues had reported the existence of PPAR γ -response elements in the upstream region of the FGF21 gene to enhance its expression, the authors found that the FGF21 knockout in mice showed defects in PPAR γ signaling (28). Therefore, we can postulate that FGF21, as a crucial mediator of PPAR γ signal transduction, has a connection with development of hypertension at least in part via retention of fluid (27). However we still didn't know the association between neuronal system controlling blood pressure and FGF21 signaling.

Limitations of our study were that the study design was cross-sectional with potential selection bias. In addition, our results had low correlation coefficients.

In conclusion, FGF21 levels are associated with serum triglyceride levels and systolic BP. Further cohort studies could determine the usefulness of serum FGF21 levels as a biomarker for some metabolic disorders associated with metabolic syndrome.

CONFLICTS OF INTEREST

We declare that the presenting authors have no conflicts of interest.

ACKNOWLEDGEMENTS

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