Fibroblast Growth Factor-19 Levels in Type 2 Diabetic Patients with Metabolic Syndrome

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Abstract. This study aimed to examine fibroblast growth factor-19 (FGF-19) in type 2 diabetic (T2DM) patients with metabolic syndrome (MetS) and to evaluate the relationship between FGF-19 and other cardiovascular risk factors, such as atherogenic index of plasma (AIP) and hsCRP. 26 T2DM patients with MetS and 12 healthy controls were enrolled in the study. Serum FGF-19 levels were measured by sandwich ELISA, and compared with other cardiovascular risk factors; lipid profile, AIP, glucose, HbA1c, and hsCRP. AIP was calculated as log (TG/HDL-c). The median (1-3.quartile) FGF-19 levels in T2DM patients with MetS and healthy controls were 122.90 (108.63-237.60) pg/ml and 293.45 (153.64-370.31) pg/ml, respectively (P=0.003). Patients were also grouped by body mass index (BMI) <30 kg/m² (n=13) and ≥30 kg/m² (n=13) with median (1-3.quartile) FGF-19 values 168.70 (113.54-275.77) pg/mL and 115.89 (97.94-200.40) pg/mL, respectively (P=0.007). Significant negative correlations were found between FGF-19 and BMI, triglyceride, log (TG/HDL-c), hsCRP, and HbA1c (r=-0.526, P=0.001; r=-0.327, P=0.05; r=-0.312, P=0.05; r=-0.435, P=0.006; r=-0.357, P=0.028, respectively). We showed that FGF-19 levels are low in T2DM patients with MetS. The negative relationship between FGF-19 and several known cardiovascular risk factors such as TG, log (TG/HDL-c), hsCRP and HbA1c in diabetic patients with MetS suggests that FGF-19 can be used as a contributing marker.

Key words: fibroblast growth factor-19; type 2 diabetes mellitus; metabolic syndrome; cardiovascular risk factor

Introduction

The metabolic syndrome (MetS) refers to the concurrence of several known cardiovascular risk factors, including insulin resistance, obesity, atherogenic dyslipidemia, and hypertension [1]. Individuals with MetS have an increased burden of cardiovascular disease (CVD) [2, 3]. In addition to their effect on cardiovascular morbidity and mortality, the components of MetS have been associated with diabetes. In the Botnia study, in women and men, respectively, MetS was seen in 10% and 15% of participants with normal glucose tolerance, 42% and 64% of those with impaired fasting glucose/impaired glucose tolerance, and 78% and 84% of those with T2DM [4].

Insulin resistance and MetS are associated with certain lipid disturbances, including high fasting and postprandial levels of triglyceride (TG) as well as instability in high-density lipoprotein cholesterol (HDL-c) concentrations and non-HDL cholesterol levels [5]. Atherogenic dyslipidemia is generally characterized by increased plasma TG and decreased HDL-c. Since the major focus is on the connection between lipids and coronary heart disease; the Adult Treatment Panel III has recognized the important roles of low HDL-c and high TG as atherogenic dyslipidemia in MetS [6]. Dobiasova and Frohlich proposed the term atherogenic index of plasma (AIP), defined as log (TG/HDL-c), on the basis that individuals with a high AIP are at a higher risk for coronary heart disease than those with a low AIP [7]. The simultaneous use of TG and HDL-c as AIP may be useful for predicting plasma atherogenity [8].
In MetS, attention has mostly focused on the vascular endothelium, where low-grade inflammatory processes lead to a continuum of vascular insults ranging from early endothelial dysfunction to advanced atherosclerosis. Inflammatory cytokines and proteins such as C-reactive protein (CRP) have been suspected to be both markers and mediators of oxidative stress and endovascular toxicity. Numerous studies have confirmed that CRP levels are elevated in individuals with MetS, and it has been proposed that high sensitive CRP (hsCRP) should be added as a clinical criterion for MetS [9].

Fibroblast growth factors (FGFs) are a family of more than 20 peptides initially characterized by their ability to stimulate fibroblast proliferation through FGF receptors (FGFR1, 2, 3, 4) [10]. Although FGFs are widely appreciated as differentiation factors, it has become apparent that the biology of FGFs is more complex and participates in the maintenance of physiological homeostasis. FGF-19, a peptide with 216 amino acids, including a signal peptide of 22 amino acids [11], was recently introduced as a novel metabolic regulator reversing diabetes mellitus, hepatic steatosis, hyperlipidemia, and adiposity [12]. The physiological function of FGF-19 is not fully understood. It has been shown that FGF-19 inhibits the expression of a cytochrome P450 enzyme, cholesterol 7α-hydroxylase (CYP7A1), which catalyzes the first and the rate-limiting step of bile acid biosynthesis from cholesterol in the liver [13]. This repression of CYP7A1 results in decreased synthesis of bile acids. An initial study by Nishimura et al indicated that FGF-19 was expressed only in the brain [14], but it has been shown that postprandial trans-intestinal bile acid flux induces the expression of FGF-19 [11, 15]. Consequently, there seems to be a negative feedback system for the synthesis of bile acid through FGF-19.

The concept of bile acid metabolism, including bile acid-activated receptors and various effecting factors, has dramatically evolved from the idea of digestive detergents to the modulation of a variety of metabolic processes through the activation of various signaling pathways. Bile acids regulate their own synthesis as well as triglyceride, cholesterol, glucose, and energy metabolism [15]. Reduced body weight, plasma levels of TG, glucose, and induced energy expenditure were observed in mice over expressing or treated with FGF-19, in addition to its influence on bile acid metabolism [12, 16, 17].

In the view of these data, we postulated that serum FGF-19 levels could be associated with several known cardiovascular risk factors such as atherogenic dyslipidemia, BMI and hsCRP in T2DM patients with MetS.

In this pilot study, we aimed to examine FGF-19 levels in T2DM patients with MetS and to evaluate the relationship between FGF-19 and other cardiovascular risk factors such as AIP and hsCRP.

Materials and Methods

Patients. The study included 26 T2DM patients with MetS and 12 healthy control subjects. All patients were clinically assessed by detailed history (including age, duration of diabetes, medication, and vitamin supplementation) and physical examination. T2DM was diagnosed according to the American Diabetes Association criteria [18]. The inclusion criterion was the diagnosis of MetS according to the American Health Association/National Heart Lung Blood Institute (AHA/NHLBI) [19]. Since all patients were Type 2 diabetic, MetS was identified as the presence of two or more of the following components: 1) WC ≥102 cm in men and ≥88 cm in women; 2) TG ≥1.7 mmol/L, or specific treatment for this lipid abnormality; 3) HDL-c <1.03 mmol/L in men and <1.29 mmol/L in women, or specific treatment for this lipid abnormality; 4) SBP/DBP ≥130/85 mmHg, or specific treatment of previously diagnosed hypertension.

Exclusion criteria were previous acute myocardial infarction, coronary bypass surgery or coronary angioplasty, chronic hepatic disease, malabsorption syndrome, diarrhea, chronic renal insufficiency, cancer, alcohol abuse, acute-chronic infectious disease, pregnancy, and any treatment related to bile acid and lipids.

The participants’ height and weight were routinely measured. Body mass index (BMI) was calculated as body weight/height² (kg/m²). The subjects were all informed about the protocol and their informed consents were obtained.

Blood sampling. Blood samples were drawn from each participant following an overnight fast of 12 hours. Venous blood samples were drawn into clot-activated tubes and were centrifuged at 3000g for 10 min; the
sera for FGF-19 measurement was stored at -80°C, until the measurement was performed. All samples were analyzed within 3 months of inclusion in the study.

**Laboratory investigation.** Serum fasting glucose, lipid profile (total cholesterol, HDL-c and TG), hsCRP, and whole blood glycated hemoglobin (HbA1c) levels were measured on the Roche Modular DPP chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula. The AIP was calculated as log (TG/HDL-c), with TG and HDL-c expressed in molar concentrations [7].

**FGF-19 measurement.** The human FGF-19 was measured in duplicate by sandwich enzyme-linked immunosorbent assay (Biovendor, Human and Diagnostic Products, Cat no: RD191107200R, Czech Republic). The manufacturer declared the within-run and between-run coefficient of variations as 7.0% and 8.5%, respectively, with sensitivity 4.8 pg/mL, and linearity 800 pg/mL. Organon Teknika Microwell system, Reader 230s (Germany) ELISA reader was used.

**Statistical analysis.** Data management was carried out with the statistical program SPSS version 17 (Statistical Package for the Social Sciences Inc. Chicago IL, USA) for Windows. Two-tailed P-values <0.05 were considered statistically significant. The Kolmogorov-Smirnov test was applied to both groups to determine the concordance with the Gaussian distribution of variables. Since none of the groups showed a Gaussian distribution, non-parametric statistics were applied. For descriptive statistics, median (1st-3rd quartile) values were given. The Mann-Whitney U-test was performed to compare variables between T2DM patients with MetS and control groups. The multiple stepwise linear regression analysis was used to identify those variables with the strongest associative influence on FGF-19. The degree of association between FGF-19 and other variables was determined using the Spearman rank correlation test.

**Results**

Age, BMI, gender, blood pressure, lipid profile, glucose, and hsCRP values of patients and healthy controls are given in Table 1. The median (1st - 3rd quartile) values of FGF-19 in the two groups were 122.90 (108.63 - 237.60) pg/mL and 293.45 (153.64 - 370.31) pg/mL, respectively (Figure 1).

There was a significant difference between the two groups (P=0.003). Although median FGF-19 levels were lower in females [116.81 (106.80-182.71) pg/mL] than in males [184.01 (111.44-271.86) pg/mL] in the patient group, this difference was not

### Table 1: Clinical and biochemical characteristics of the T2DM patients with MetS and controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients (n=26)</th>
<th>Controls (n=12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54 (46.75-60.25)</td>
<td>49.5 (38-53.75)</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Gender (male %)</td>
<td>46%</td>
<td>45%</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.9 (26.2-31.3)</td>
<td>22.6 (21.4-23.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>130 (127.5-140)</td>
<td>110 (100-120)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80 (78-90)</td>
<td>72.5 (70-80)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.93 (5.93-8.71)</td>
<td>4.81 (4.68-4.99)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6 (6-8)</td>
<td>5 (4.5-5)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.62 (4.09-5.51)</td>
<td>4.60 (3.88-5.04)</td>
<td>0.695</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.51 (1.04-2.31)</td>
<td>0.94 (0.61-1.16)</td>
<td>0.004*</td>
</tr>
<tr>
<td>HDL-c (mmol/L)</td>
<td>1.18 (0.98-1.43)</td>
<td>1.50 (1.34-1.83)</td>
<td>0.002*</td>
</tr>
<tr>
<td>LDL-c (mmol/L)</td>
<td>2.90 (2.13-3.29)</td>
<td>2.68 (2.0-3.17)</td>
<td>0.346</td>
</tr>
<tr>
<td>Log (TG/HDL-c)</td>
<td>0.79 (0.55-0.99)</td>
<td>0.39 (0.28-0.63)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>2.62 (1.06-6.9)</td>
<td>0.86 (0.52-1.56)</td>
<td>0.006*</td>
</tr>
<tr>
<td>FGF-19 (pg/mL)</td>
<td>122.90 (108.63-237.60)</td>
<td>293.45 (153.64-370.31)</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

**BMI:** body mass index, **SBP:** systolic blood pressure, **DBP:** diastolic blood pressure, **HbA1c:** glycated hemoglobin, **TC:** total cholesterol, **TG:** triglyceride, **HDL-c:** high density lipoprotein-cholesterol, **LDL-c:** low density lipoprotein-cholesterol, **Log (TG/HDL-c):** logarithm of molar triglyceride/molar HDL-c, **hsCRP:** high sensitive C-reactive protein, **FGF-19:** fibroblast growth factor-19.

Data are shown as median (1st-3rd quartile), *P<0.05 is significantly different between patients and controls.
FGF-19 levels in type 2 diabetics with metabolic syndrome

FGF-19 levels in type 2 diabetics with metabolic syndrome statistically different ($P=0.237$). Furthermore, there were no significant differences in terms of clinical and biochemical characteristics between males and females in the patient and control groups (data not shown).

There was a moderate negative correlation between FGF-19 and age ($r=-0.427, P=0.008$), BMI ($r=-0.526, P=0.001$) and with some other laboratory data. The correlations between laboratory variables are given in Table 2.

A multiple stepwise regression was performed to determine which variables had the strongest association with FGF-19 concentration. Age, gender, blood pressure, BMI, total cholesterol, HDL-c, LDL-c, TG, log (TG/HDL-c), fasting glucose, and HbA1c were the independent variables. All variables except BMI were excluded. The $R$ for regression (0.445) was significantly different from zero ($P=0.005$), with $R^2$ at 0.198 indicating that approximately 20% of the variability in FGF-19 is predicted by BMI.

The patients were also grouped according to their BMI as <30 kg/m$^2$ (n=13) and ≥30 kg/m$^2$ (n=13). The median (1st -3rd quartile) values of FGF-19 were 168.70 (113.54- 275.77) pg/mL and 115.89 (97.94- 200.4) pg/mL, respectively, and they were significantly different ($P=0.007$) (Figure 2).

Discussion

FGF-19 is a new potent metabolic regulator that influences glucose and lipid homeostasis [20]. The physiological function of FGF-19 has not been resolved, but Holt et al showed that hepatocyte expression of FGF-19 is induced by transcription factor, farnesoid X receptor (FXR) [13]. The bile acids that represent the primary pathway for cholesterol catabolism in the liver [15] were identified as the natural ligands for the FXR, a “bile acid sensor”. FXR induces the expression of FGF-19. FGF-19 inhibits the hepatic CYP7A1, which catalyzes the first and the rate-limiting step of bile acid biosynthesis from cholesterol in the liver [13]. The net result of FXR activation is the repression of bile acid production. FXR regulates the metabolism not only of bile acid, but also of cholesterol, lipoprotein, triglyceride, and glucose. Cholesterol, triglyceride, and glucose dysregulations give rise to the MetS including T2DM, obesity, dyslipidemia, and atherosclerosis [21].

Tomlinson et al showed that FGF-19 transgenic mice have a significant increase in metabolic rate and are resistant to diet-induced obesity [16]. Fu et al reported that recombinant FGF-19 increased metabolic rate, reduced body weight, and reversed the diabetes in both high-fat-fed mice and leptin-deficient mice. Injection of FGF-19 into the lateral

<table>
<thead>
<tr>
<th>Variable</th>
<th>FGF-19 (pg/mL) r, P</th>
<th>Log (TG/HDL-c) r, P</th>
<th>hsCRP (mg/L) r, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>$r=-0.526, P=0.001^*$</td>
<td>$r=0.499, P=0.001^*$</td>
<td>$r=0.344, P=0.035^*$</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>$r=-0.260, P=0.115$</td>
<td>$r=0.430, P=0.007^*$</td>
<td>$r=0.297, P=0.070$</td>
</tr>
<tr>
<td>HDL-c (mmol/L)</td>
<td>$r=0.163, P=0.329$</td>
<td>---</td>
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</tr>
<tr>
<td>TG (mmol/L)</td>
<td>$r=-0.327, P=0.05^*$</td>
<td>---</td>
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</tr>
<tr>
<td>Log (TG/HDL-c)</td>
<td>$r=-0.312, P=0.05^*$</td>
<td>---</td>
<td>$r=0.444, P=0.005^*$</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>$r=-0.435, P=0.006^*$</td>
<td>$r=0.444, P=0.005^*$</td>
<td>$r=0.502, P=0.001^*$</td>
</tr>
<tr>
<td>HbA1c</td>
<td>$r=-0.357, P=0.028^*$</td>
<td>$r=0.502, P=0.001^*$</td>
<td>$r=0.510, P=0.001^*$</td>
</tr>
</tbody>
</table>

*: moderate correlation, ---: variable part of the logarithm, r: coefficient of correlation.

If $r$ is between 0.25 and 0.50, there is moderate correlation and if $r$ is greater than 0.50, there is good correlation between the variables.
ventricle of the brain also increased metabolic rate. The authors suggested that FGF-19 increased lipid oxidation and decreased expression of acetyl coenzyme A carboxylase 2, and as a direct consequence, carnitine palmitoyl transferase 1 activity and fatty acid oxidation increase [17]. Under these conditions, metabolic rate increases and a resistance to diet-induced obesity develops [16]. Therefore, FGF-19 might improve dyslipidemia, hyperinsulinemia, hyperleptinemia, and insulin sensitivity and reduce body weight and adiposity [20].

In accordance with previously published data, we showed that the serum FGF-19 levels of diabetic patients with MetS were significantly lower than those of the healthy control subjects. Additionally, in our study, the serum TG, HDL-c, and log (TG/HDL-c) also differed significantly between the two groups. Stejskal et al demonstrated the relationship between FGF-19 levels and glucose, HDL-c, and TG levels, but they found no significant correlation between FGF-19 and BMI [20]. In contrast to their findings, we showed that FGF-19 and BMI were negatively correlated, but no correlations existed between FGF-19 and glucose or HDL-c. We also found that FGF-19 was significantly lower in MetS patients with BMI greater than 30 kg/m² than patients with BMI less than 30 kg/m². In addition, there was a negative correlation and linear regression between FGF-19 and BMI. That is, as obesity is a component of MetS, BMI increase results in FGF-19 decline. FGF-19 selectively binds to FGFR4 and it does not lead to a fibroblastic proliferation. Recently, new evidence suggesting that FGFR4 is also involved in phenotypes related to the MetS has come to light. For example, FGFR4-deficient mice that were fed a regular diet displayed hyperlipidemia, glucose intolerance, and insulin resistance as well as increased weight gain compared with wild-type littermates. Restoration of FGFR4 in the livers of FGFR4-deficient mice decreased plasma lipid levels [22]. In the study of Shin et al, FGFR4 has also been implicated in insulin regulation that regulates glucose and lipid metabolism in the liver and adipocyte, as a key node in integrating the FGF-19 and insulin signaling pathways [23]. In our study, the negative correlation between FGF-19 and HbA1c also supports the relationship between FGF-19 and glucose homeostasis. Since our patient group consisted of Type 2 diabetics, finding no significant correlation between fasting glucose and FGF-19 was an expected result, as HbA1c is a better indicator of glucose homeostasis; moreover, all patients were on anti-diabetic medication. On the other hand, HbA1c has also been known to predict vascular complications, including cardiovascular disease, in diabetic patients. This situation suggests that better control of HbA1c concentrations is associated with a reduction in cardiovascular events [24].
Selvin et al published a meta-analysis of 13 prospective cohort studies related to glycated hemoglobin and cardiovascular disease in diabetic patients. The estimated risk of cardiovascular disease increased by 18% for each 1% increase in absolute glycated hemoglobin value [25]. According to the aforementioned data, the correlation between HbA1c and FGF-19 supports the possibility that FGF-19 may be another prominent CVD risk factor.

In MetS patients, obesity is a potent factor that contributes to insulin resistance, which plays a major role in the pathogenesis of CVD. Obesity influences insulin action and promotes atherogenic dyslipidemia through the release of inflammatory cytokines, leptin, TNF-α, and IL-6. An important manifestation of high levels of cytokines is elevated plasma levels of CRP [26, 27]. Several studies have demonstrated relationships between CRP and individual components of the MetS [28, 29]. Yudkin et al found a significant correlation between inflammatory markers and several features of the MetS. CRP levels were shown to be strongly associated with insulin resistance (calculated from the homeostatic model assessment), blood pressure, HDL-c, and TG in a study of 107 non-diabetic individuals. BMI and insulin resistance were the strongest determinants of the inflammatory state [30]. Furthermore, in the Insulin Resistance and Atherosclerosis Study (IRAS), Festa et al showed that hsCRP was positively correlated with BMI, waist circumference, blood pressure, TG, total cholesterol, LDL-c, plasma glucose, and fasting insulin and inversely correlated with HDL-c and the insulin sensitivity index in non-diabetic people [31].

In our study, T2DM patients with MetS had significantly higher hsCRP than healthy controls. hsCRP was negatively correlated with FGF-19 and positively correlated with BMI, TG, log (TG/HDL-c), and HbA1c. Mild chronic elevations of hsCRP concentrations are independently predictive of future cardiovascular events [32, 33]. Additionally, the joint occurrence of elevated TG and low HDL-c levels were viewed to define the affection status of atherogenic dyslipidemia since they are primary features associated with insulin resistance detectable early in the development of the MetS, individually highly inherited, and relatively simple to quantify. Dyslipidemia is also an important risk factor for the development of CVD and treatment approaches to decrease LDL-c levels have reduced cardiovascular events. Recent analyses demonstrate that hypertriglyceridermia is an independent predictor of CVD and may be a risk factor [34]. FGF-19 increases fatty acid oxidation, which leads to a decrease in TG concentrations. In diabetic patients with MetS, FGF-19 concentrations were low and decreased fatty acid oxidation resulted with atherogenic effect. The ratio of TG/HDL-c has been proposed as an easily obtainable atherogenic marker. Recently, Dobiásová et al showed that log (TG/HDL-c) has a place in the assessment of lipid-related risk for CVD and that this index can be used in general practice as an alternative marker of plasma atherogenity [7]. We showed that low FGF-19 levels were closely related with this atherogenity marker.

There are several limitations in our study. This is a pilot study with small number of patients and controls. Since prospective cohort studies are needed, FGF-19 cannot yet be pronounced an independent risk assessment marker or a surrogate of other markers. Further investigations in diabetic patients with or without MetS should be performed to determine whether FGF-19 can be used in predicting cardiovascular disease risk. Additionally, the relationship between FGF-19 and insulin resistance could be investigated. Nevertheless, our results elucidate valuable clues about FGF-19, a potent regulator of bile acid metabolism, and its interactions with known cardiovascular risk factors.

In conclusion, this study shows that serum FGF-19 levels are low in T2DM patients with MetS. The negative relationship between FGF-19 and several known cardiovascular risk factors such as TG, log (TG/HDL-c), hsCRP, and HbA1c in diabetic patients with MetS suggests that FGF-19 can be used as a contributing marker.
References


