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The U-shaped relationship between fibroblast growth factor 21 and microvascular complication in type 2 diabetes mellitus

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ABSTRACT

Aims: The aim of this study was to investigate the relationship between serum FGF21 level and all microvascular complication including cardiac autonomic neuropathy (CAN) in patients with type 2 diabetes mellitus (T2DM).
Methods: A total 227 T2DM patients were enrolled and serum FGF21 levels were assessed. Diabetic retinopathy, nephropathy, peripheral neuropathy (DPN), and CAN were evaluated.
Results: The prevalence of retinopathy and nephropathy among the FGF21 tertiles was significantly different ($p = 0.001$, $p = 0.006$, respectively), whereas no difference was found in the prevalence of DPN and CAN. In multivariate analysis, the odds ratio (OR) for the presence of retinopathy was 0.08 for the FGF21 second tertile when compared with the first tertile ($p = 0.029$). OR of retinopathy in third tertile group was lower than first tertile and higher than second tertile, but statistically insignificant. Crude OR for nephropathy was 0.34 for the second FGF21 tertile, when compared with the first tertile ($p = 0.015$). However, FGF21 level was not significantly associated with nephropathy after multivariable adjustment.
Conclusions: In the present study, there was no association between diabetic nephropathy, DPN, and CAN and serum FGF21 levels. However, we found a U-shaped relationship between both lower and higher serum FGF21 levels and diabetic retinopathy. This result suggests that the very low serum FGF21 level itself may associate with diabetic retinopathy and also relatively elevated serum FGF21 level may be a compensatory increase to protect against microvascular injury.

1. Introduction

Fibroblast growth factor (FGF21), a 181 amino acid circulating protein, metabolic hormone predominantly produced by the liver, is also expressed in adipocytes and the pancreas (Kharitonenkov et al., 2005). It regulates glucose and lipid metabolism through the interaction with specific FGF receptors and a cofactor called beta-Klotho (Kharitonenkov et al., 2008). It exerts various favorable metabolic effects in rodents and diabetic monkeys (Coskun et al., 2008). Potential utility of FGF21-based pharmacotherapy in non-human primates and obese humans with T2DM has been suggested (Gimeno & Moller, 2014).

Nonetheless, in human studies, high serum FGF21 levels are found in obesity, T2DM, metabolic syndrome (MS), non-alcoholic fatty liver disease (NAFLD), and coronary artery disease (CAD) (Bobbert et al., 2013; Dushay et al., 2010; Lin et al., 2010; Xiao et al., 2012). Although these paradoxical phenomena have been considered as FGF21 resistance or compensatory increase to underlying metabolic stress, the exact pathophysiologic roles of FGF21 in human are unclear yet (Kim & Lee, 2014; Woo, Xu, Wang, & Lim, 2013).

Most previous studies were performed to evaluate whether serum FGF21 levels are associated with metabolic parameters, cardiovascular risk factors, adiposity index, and atherosclerosis in diverse populations (Akyildiz et al., 2015; Chow et al., 2013; Lin et al., 2010). In T2DM subjects, a few studies were reported regarding the association between elevated serum FGF21 levels and carotid atherosclerosis (An et al., 2012; Xiao et al., 2015). In addition, only very limited clinical studies regarding associations between serum FGF21 levels and diabetic microvascular complication such as retinopathy, nephropathy, and peripheral neuropathy have been reported (Jian et al., 2012; Lee et al., 2015; Lin et al., 2014; Ong et al., 2015).

Very recently, FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study in patients with T2DM revealed that higher baseline serum FGF21 levels are seen in patients with baseline total microvascular disease and predict the future development of new microvascular disease (Ong et al., 2015). Beyond that several studies

The authors declare that there is no conflicting of interest.

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have investigated the association between serum FGF21 levels and diabetic nephropathy (An et al., 2012; Jian et al., 2012; Lee et al., 2015). Whereas some studies suggested that elevated serum FGF21 levels were correlated with presence of nephropathy, another study did not show any difference in FGF21 levels according to the presence or absence of diabetic nephropathy. In addition, only four studies including FILED study have investigated the association between serum FGF21 levels and diabetic retinopathy (An et al., 2012; Esteghamati et al., 2016; Lin et al., 2014; Ong et al., 2015). However, these studies showed inconsistent results. Moreover, to our knowledge, there is no report in the literature on the association of FGF21 levels and CAN.

It is now well recognized that the visceral adipose tissue confers a risk of metabolic and CV complications (Wajchenberg, 2000). Previous two studies have reported that serum FGF21 and its association with epicardial fat thickness using echocardiography or pericardial fat area by multidetector computed tomography (Akyildiz et al., 2015; Lee et al., 2014). However, there are limited data of FGF-21 such as its association with ectopic (visceral) fat depots. To the best of our knowledge, no prior reports have been published regarding the relationship between FGF21 and abdominal fat thickness using ultrasonography (US).

The purpose of this study, therefore, was to evaluate the relationship between serum FGF21 levels and all microvascular complication including CAN, and abdominal fat thickness in patients with T2DM.

2. Materials and methods

2.1. Study design and subjects

A total 256 patients with T2DM who were admitted at Endocrinology Division of Soonchunhyang University Bucheon Hospital, from January 2014 to July 2015 were screened for the study. Patients were excluded if they had type 1 diabetes, or older than 80 years of age, taking fibrates or were not available of clinical data from appropriate medical records and fasting serum samples. Finally, this study was performed on 227 T2DM patients (114 men and 113 women; mean age: 53.7 years). We reviewed detailed demographic data, biochemical data, clinical and treatment history using medical records. Among subjects with hypoglycemic medications, subjects taking thiazolidinedione (TZD) (all was pioglitazone) were 15 and taking metformin were 111. There was no subject prescribed GLP-1 agonists. The smoking status of the subjects was classified as being a non-smoker or current smoker. The alcohol consumption of the participants was classified as 'yes' or 'no'. All patients were informed of the purpose of the study, and their consent was obtained. The study was approved by the Institute Review Board of Soonchunhyang University College of Medicine, Bucheon Hospital.

2.2. Anthropometric and biochemical measurements

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (square meters).

Blood samples were taken after overnight fasting. Fasting plasma glucose (FPG), C-peptide levels, HbA1c, and lipid profiles were measured. HbA1c was measured by ion-exchange high-performance liquid chromatography (Bio-Rad, Hercules, CA, USA). Serum C-peptide was measured using an immunoradiometric assay kit (Immunotech, Prague, Czech Republic). The IR status was evaluated by the homeostasis model assessment–insulin resistance (HOMA-IR) index. The HOMA-IR was calculated by the following formula: [fasting insulin (uIU/mL) × fasting blood glucose (mmol/L)]/22.5. The HOMA-IR score was available only in 147 patients not receiving exogenous insulin.

Blood samples were taken after overnight fasting and stored continuously at −80 until the time of the analysis of serum FGF21. Serum FGF21 was measured using ELISA (quantikine human FGF21 ELISA; R&D Systems, Minneapolis, MN). The assay has a minimum limit of detection of 4.6 pg/mL. No samples were below the lower limit of detection.

2.3. Evaluation of diabetic microvascular complications

Diabetic retinopathy was evaluated by experienced ophthalmologists, while the patients' pupils were dilated. If needed, fluorescein angiography was performed. Diabetic retinopathy was classified with five stages as normal, nonproliferative (mild, moderate, and severe), and proliferative retinopathy based upon the findings of the Wisconsin Epidemiologic Study of Diabetic Retinopathy and the Early Treatment of Diabetic Retinopathy Study (Wilkinson et al., 2003). Patients were considered to have retinopathy if they show nonproliferative or proliferative stage.

Diabetic nephropathy was defined using albuminuria, which was measured by radioimmunoassay (Immunotech). Albumin excretion rate (AER) of <20 μg/min or urine albumin of <30 mg/g creatinine was categorized as normoalbuminuria, AER in the range of 20–200 μg/min or urine albumin of 30–300 mg/g creatinine was categorized as microalbuminuria and AER of >200 μg/min or urine albumin of ≥300 mg/g creatinine was categorized as overt proteinuria. Patients were considered to have nephropathy if they show microalbuminuria or overt proteinuria. Estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease (MDRD) study equation.

Diabetic peripheral neuropathy (DPN) was diagnosed with recommendation by the Expert Committee of Korean Diabetes Neuropathy Study Group, as: the presence of typical symptoms using the Michigan Neuropathy Screening Instrument (MNSI) and compatible findings on neurologic screening examinations or electrophysiologic studies (Feldman et al., 1994; Won, Kim, Ko, & Cha, 2014). Although electrophysiological studies are not essential, current perception threshold (CPT) test was performed in all patients using a Neurometer CPT/C (Neurotron, Baltimore, MD).

Cardiac autonomic neuropathy (CAN) was assessed by autonomic function test (AFT). CAN was assessed by the five standard cardiovascular reflex tests according to the Ewing's protocol (Ewing, Martyn, Young, & Clarke, 1985). The severity of CAN was quantitated by summation of points obtained from each of the five tests, where each test was given a point of 0, 0.5, or 1 if it yielded a normal, borderline, or abnormal value, respectively. CAN was defined as the presence of at least two abnormal tests or an autonomic neuropathy points ≥ 2 (Bellavere, Bosello, Fedele, Cardone, & Ferri, 1983).

Sonographic measurement of abdominal fat thickness was performed using a high resolution B-mode US by a single experienced investigator. Subcutaneous fat thickness (SFT) and visceral fat thickness (VFT) were measured in the region 1 cm above the umbilicus using a 12-MHz linear-array probe and a 3.5-MHz convex-array probe, respectively. SFT was defined as the maximal thickness of the fat tissue layer between the skin-fat interface and the linea alba. VFT was defined as the distance between the anterior wall of the aorta and the posterior aspect of the rectus abdominis muscle perpendicular to the aorta (Kawamoto, Ohtsuka, Ninomiya, & Nakamura, 2008; Kim et al., 2004; Stolk et al., 2001). The intra-observer technical error of measurement for the VFT was between 1.4%–2.3% and 1.1%–1.7% for the SFT.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 14.0 (SPSS Inc., Chicago, IL, U.S.A). Data are reported as mean ± standard deviation (SD) for normally distributed variables, as median (interquartile

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