Int J Clin Exp Pathol 2016;9(9):9625-9630
www.ijcep.com /ISSN:1936-2625/IJCEP0035246

Original Article

Decreased levels of serum fibroblast growth factor 21 in Chinese patients with coronary artery disease

Hai-Feng Chen, Feng Wu, Nan Lu, Wei-Ping Zheng, Kai-Yang Lin

Department of Cardiology, Fujian Provincial Clinical College, Fujian Medical University, Fuzhou, Fujian, China

Received July 7, 2016; Accepted July 20, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: Objective: The purpose of this study was to examine the relationship between serum FGF21 and coronary artery disease (CAD) and to explore the significant role of FGF21 in CAD patients. Methods: A total of 405 Chinese patients were recruited for this study between June 2013 and June 2014, including 252 patients with CAD and 153 patients without CAD. The levels of serum FGF21 was measured by ELISA. The statistically independent risk factors for CAD were identified by multivariate logistic regression analysis. Results: In the Spearman correlation analysis, serum FGF21 levels correlated negatively with coronary artery disease ($r=-0.116$, $P<0.05$). Univariate logistic regression analysis revealed a significant correlation between CAD and age ($P=0.022$), sex ($P<0.001$), smoker ($P<0.001$), fasting plasma glucose ($P=0.001$) and HbA1c ($P<0.001$). Also, as expected, level of FGF 21 appeared to be a negatively correlated with CAD ($\beta=-0.930$; $P=0.008$). Serum FGF21 levels correlated negatively with the severity of coronary artery stenosis, including the Gensini score ($r=-0.076$, $P<0.05$) and the lesion number in the coronary artery ($r=-0.110$, $P<0.05$). Conclusion: FGF21 was shown to be one of the independent protective factors for CAD. The level of serum FGF21 correlated negatively with the severity of coronary artery stenosis, including the Gensini score and the lesion number in the coronary artery. FGF21 showed a possible protective effect on patients with coronary heart disease.

Keywords: Serum fibroblast growth factor 21, coronary artery disease

Introduction

The family of fibroblast growth factors (FGFs) is composed of hormone-like factors that modulate a number of metabolic processes, including cell proliferation, development and hyperplasia, both in vitro and in animal models. Among the endocrine subfamily, great attention has been paid to FGF19, FGF21 and FGF23, especially to their roles in regulating glucolipide metabolism. Many studies have shown that FGF21 is becoming a new candidate drug for the therapy of diabetes. FGF21 not only modulates glucose and lipid homeostasis [1, 2] but also plays an important role in the processes of endothelial cell apoptosis [3, 4] and myocardial ischemia protection [5, 6]. Therefore, FGF21 may play a significant role in the occurrence and development of coronary heart disease. Large recent studies have shown that FGF21 may have some correlation between the occurrence and development of coronary heart disease [7, 8]. Therefore, the main aim of our study was to explore the relation between the serum levels of FGF21 and coronary artery disease occurrence and the severity of coronary artery lesions in patients with CAD and to assess the correlation between FGF21 and other clinical variables.

Materials and methods

Study population

Four hundred and five Chinese patients visiting the Department of Cardiology in the Fujian Provincial Hospital who underwent coronary arteriography were recruited for our study between June 2013 and June 2014. Exclusion criteria were the presence of hypo- or hyperthyroidism, acute or chronic viral hepatitis and kidney diseases, drug-induced or alcoholic liver diseases, alcoholism, total parenteral nutrition, current or long-term therapies with systemic glucocorticoids or tumor.

The local Ethics Committee of Fujian Provincial Hospital approved this study, and all of the enrolled subjects provided informed consent.

Anthropometric and biochemical parameters

Base characteristics, including weight, height, hip circumference (HP), waist circumference (WC) and blood pressure (BP), were measured. Waist/hip ratio (WHR) and body mass index (BMI) were also calculated. Smoking status was also recorded.

Twelve hours after hospital admission, blood samples were obtained in the Department of Clinical Laboratory. The concentrations of total cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and apolipoprotein (a) and glucose were measured using a Cobas 8000 biochemical autoanalyzer (Roche, Tokyo, Japan). Glycated hemoglobin A1c (HbA1c) was measured by liquid chromatography using an HA-8180 automatic glycosylated hemoglobin analyzer (ARKRAY, Tokyo, Japan). Fasting serum insulin was assessed by the E601 automatic electrochemiluminescence analyzer (Roche, Tokyo, Japan). Insulin resistance (IR) was calculated by HOMA-IR model: [fasting glucose (mmol/L) × fasting insulin (mU/L)]/22.5.

Quantification of fibroblast growth factor 21

Plasma was collected from the patient in a fasting state on the morning after hospitalization. Blood was collected and immediately processed. Peripheral blood was centrifuged at 4000 rpm for 10 minutes. Serum was collected and stored at -70°C until analysis. Serum concentrations of FGF21 were quantified using human ELISA kits with a biotin-labeled antibody (Catalog number RD191108200R, Biovendor Laboratory Medicine, Inc., Brno, Czech Republic). The intra-assay variation among the duplicates was <4%. All of the inter-assay coefficients of variation (CV) were <5%.

CAD diagnosis and other diagnostic criteria

The standard Judkins technique was carried out for arteriography and three major coronary arteries were examined carefully at least twice. The arteriography analysis was randomly carried out by more than one interventional operators who were blinded to this study protocol. A patient was considered to have CAD when a stenotic lesion resulting in a 50% or greater reduction in lumen diameter existed in at least one of the coronary arteries. The severity of coronary artery stenosis was evaluated by Gensini scores [9]. According to the number of stenotic lesions of coronary arteries detected ≥ 50%, we categorized the arteries as single-vessel lesion, double-vessel lesion and multi-vessel lesion. Hypertension was defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg on two different occasions or the taking of antihypertensive medication. Diabetes was defined according to the clinical diagnosis of the physician and/or an HbA1c ≥ 6.5% and/or a fasting plasma glucose (FPG) ≥ 7.0 mmol/L and/or a 2-h plasma glucose (PG) ≥ 11.1 mmol/L during the oral glucose tolerance test (OGTT).

Statistical analysis

All analyses were used the SPSS software (version 19.0). The normality of the variables was confirmed by Kolmogorov-Smirnov and Shapiro-Wilk tests. Baseline characteristics of the participants were performed with t-test (continuous variables) or the Mann-Whitney U test (non-parametric variables). Inter-group statistical significance was determined by Pearson chi-square. The statistically independent factors for CAD occurrence was identify by multivariate logistic regression analysis. The correlation of two factors was assessed by the Spearman or Kendall correlation test. All reported values were two-tailed and considered statistically significant for $P<0.05$.

Results

Anthropometric and biochemical characteristics of the subjects

A total of 405 patients were enrolled in this study, including 252 patients with CAD and 153 patients without CAD. The mean age of the participants was 63.4±9.7 years old. The proportions of males (69.7%), smokers (72.4%) and diabetics (71.8%) were much larger in the group diagnosed with CAD than in the non-CAD group. For the established cardiovascular risk factors, the CAD group was older and had significantly higher waist-to-hip ratios (WHR), fasting plasma glucose, triglycerides and HbA1c (all $P<0.05$) and lower high-density lipoprotein (HDL)-cholesterol compared with the non-CAD group.

Table 1. Baseline characteristics of the participants

Variables	Total (N=405)	Non-CAD (N=153)	CAD (N=252)	*P* value
Sex (male) (%)	287 (70.9%)	87 (30.3%)	200 (69.7%)**	<0.001
Age (years)	63.4±9.7	62.0±9.7	64.3±9.6*	0.021
Hypertension [n (%)]	278 (68.8%)	106 (38.1%)	172 (61.9%)	0.829
Diabetes [n (%)]	124 (30.4%)	35 (28.2%)	89 (71.8%)**	0.008
Smoker [n (%)]	217 (53.6%)	60 (27.6%)	157 (72.4%)**	<0.001
BMI (kg/m^2)	24.4±3.1	24.6±3.2	24.4±3.1	0.572
WHR	0.94 (0.89-0.97)	0.91 (0.87-0.96)	0.95 (0.90-0.98)**	<0.001
SBP (mmHg)	130.0 (117.0-144.0)	132.0 (117.0-147.0)	129.0 (118.0-143.0)	0.500
DBP (mmHg)	74.0 (68.0-83.0)	75.0 (68.5-83.0)	74.0 (67.3-83.0)	0.178
Fast plasma glucose (mmol/L)	5.2 (4.8-5.9)	5.1 (4.7-5.5)	5.3 (4.8-5.2)**	0.004
Fast insulin level (mIU/mL)	7.85 (4.62-11.76)	8.07 (4.45-12.81)	7.67 (4.67-11.23)	0.335
HOMAIR	1.9 (1.1-3.1)	1.96 (0.94-3.12)	1.8 (1.2-3.1)	0.825
HbA1c (%)	6.1 (5.8-6.7)	5.9 (5.7-6.3)	6.1 (5.8-6.9)**	<0.001
TG (mmol/L)	1.2 (0.9-1.8)	1.1 (0.8-1.8)	1.3 (0.9-1.8)**	0.010
TC (mmol/L)	4.0 (3.3-4.8)	4.0 (3.3-5.0)	4.0 (3.3-4.7)	0.519
HDL-C (mmol/L)	1.1 (0.9-1.3)	1.1 (0.9-1.4)	1.0 (0.9-1.2)**	<0.001
LDL-C (mmol/L)	2.5 (1.8-3.2)	2.7 (1.8-3.6)	2.5 (1.8-3.0)	0.298
Lipoprotein (a) (mg/L)	186.1 (85.4-423.5)	185.9 (83.3-391.4)	187.1 (85.5-442.7)	0.998
FGF21 (pg/mL)	342.9 (209.2-557.2)	376.1 (247.2-588.7)	334.4 (180.8-548.6)*	0.020
Gensini score	18 (5-46)	5.0 (0-1.05)	32 (18-64)**	<0.001

Data were expressed as the mean ± SD. CAD, coronary artery disease; BMI, body mass index; WHR, Waist-to-hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HOMA-IR, the Homeostasis Model Assessment of Insulin Resistance; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FGF21, fibroblast growth factor 21. (CAD group vs. non-CAD group, *P<0.05; **P<0.01).

Table 2. Independent factors for CAD were analyzed by univariate logistic regression analysis

	β	*P* value
Age	0.024	0.022
Sex	1.071	<0.001
BMI (kg/m^2)	0.219	0.571
Waist-to-hip ratio (WHR)	6.436	0.463
Smoker	0.941	<0.001
HOMA-IR	0.058	0.314
HbA1C	0.586	<0.001
FPG	0.303	0.001
TG (mmol/L)	0.320	0.719
TC (mmol/L)	0.167	0.074
HDL-C (mmol/L)	-1.458	0.678
LDL-C (mmol/L)	0.156	0.590
#FGF21	-0.930	0.008

β: standardized regression coefficient. #Data were log-transformed before analysis.

In addition, the history of hypertension, BMI, systolic blood pressure, diastolic blood pressure, HOMA-IR, total cholesterol, low-density lipoprotein (LDL)-cholesterol and lipoprotein (a) did not differ between these two groups.

As shown in **Table 1**, serum FGF21 levels was elevated in the non-CAD group compared with the CAD group (376.1 pg/mL (247.2-588.7) vs. 334.4 pg/mL (180.8-548.6), P<0.05).

Relationship between FGF21 and CAD

In the Spearman correlation analysis, serum FGF21 levels correlated negatively with coronary artery disease (r=-0.116, P<0.05). Univariate logistic regression analysis (**Table 2**) revealed a significant correlation between CAD and age (P=0.022), sex (P<0.001), smoker (P<0.001), fasting plasma glucose (P=0.001) and HbA1c (P<0.001). Also, as expected, level of FGF 21 appeared to be a negatively correlated with CAD (β=-0.930; P=0.008).

Relationship between FGF21 and the severity of coronary artery stenosis

As shown in **Table 3**, serum FGF21 levels correlated negatively with the Gensini scores (r=-

Table 3. Kendall correlation analysis between FGF21 and the severity of coronary artery stenosis

	Correlation coefficient	*P* value
Gensini score	-0.076	0.049
Lesion number in the coronary artery	-0.110	0.011

Table 4. Relationship between FGF21 and other clinical variables

	Correlation coefficient	*P* value
Waist-to-hip ratio (WHR)	0.108	0.030
BMI (kg/m^2)	0.109	0.028
Systolic blood pressure (mmHg)	0.158	0.001
Diastolic blood pressure (mmHg)	0.128	0.010
Fast plasma glucose (mmol/L)	0.067	0.176
HOMAIR	0.144	0.004
HbA1C	0.036	0.467
TG (mmol/L)	0.216	<0.001
TC (mmol/L)	0.196	<0.001
HDL-C (mmol/L)	0.042	0.404
LDL-C (mmol/L)	0.142	0.004

BMI, body mass index; HOMA-IR, the Homeostasis Model Assessment of Insulin Resistance; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FGF21, fibroblast growth factor 21.

0.076; P<0.05) and the lesion numbers in the coronary artery (r=-0.110; P<0.05).

Relationship between FGF21 and other clinical variables

In the Spearman correlation analysis, (**Table 4**) serum FGF21 levels correlated positively with waist to hip ratio (r=0.108, P=0.030), body mass index (r=0.109, P=0.028), systolic blood pressure (r=0.158, P=0.001), diastolic blood pressure (r=0.128, P=0.010), insulin resistance index (r=0.144, P=0.004), triglyceride (r=0.216, P<0.001), total cholesterol (r=0.196, P<0.001) and low density lipoprotein cholesterol (r=0.142, P=0.004). But there was no significant correlation between FGF21 and fasting blood glucose, glycosylated hemoglobin and high density lipid protein cholesterol.

Discussion

FGF21 was isolated from the embryonic tissues of mice and first identified by Nishimura et al. in 2001 [10]. The lack of a heparin-binding domain in FGF21 allows it to act as an extracellular endocrine factor [11]. Research has shown that the expression of FGF21 was induced by oxidative LDL in Wistar rat cardiac microvascular endothelial cells (CMECs) [12]. FGF21 was able to reduce the apoptosis of CMECs and improve their endothelial function. The expression of FGF21 was inhibited by shRNA, which led to the apoptosis of CMECs and an inhibition of the development of atherosclerosis and CAD. Additionally, FGF21 was found to enhance cholesterol efflux mediated by LXRα-dependent ATP binding cassette (ABC) A1 and G1, which may have a protective effect against atherosclerosis [13]. Therefore, the anti-atherosclerosis function of FGF21 may provide both direct and indirect effects through the direct inhibition of apoptosis of the endotheliocyte and the indirect improvement of metabolism of glucose and serum lipid.

In the last few decades, several studies have demonstrated that FGF21 may play a physiological role in improving endothelial cell apoptosis and in regulating glucose and lipid metabolism at an early stage of atherosclerosis [14]. However, controversy over the exact role of FGF21 in patients with CAD still exists: some publications report it as a protective factor, whereas others claim it is not. Lin et al. found that serum FGF21 was significantly elevated in the CAD patient group and elevated more obviously in those with hypertension or diabetes mellitus [8, 15]. Multivariate logistic regression analysis found that FGF21 correlated independently with CAD occurrence. However, Lee et al. found that serum levels of FGF21 declined in the CAD patients group, but there was no statistical significance between serum FGF21 levels and CAD [16].

In our study, the serum FGF21 levels in the CAD group were lower than in the non-CAD group, which was the same trend compared with Lee et al. In addition, the Spearman correlation analysis also showed that FGF21 negatively correlated with CAD. After adjustment with the confounding variables of age, sex, smoking status, HbA1c and HOMA-IR, a multivariate logistic regression analysis showed that a significant negative association existed between FGF21 and CAD. FGF21 negatively correlated with coronary artery stenosis severity and lesion numbers in the coronary artery as well. Above all,

these data demonstrated that the level of serum FGF21 may have a protective effect against coronary artery disease and that a decrease in FGF21 was likely to trigger or exacerbate coronary artery disease.

Adiponectin is a peptide hormone that is secreted by adipose tissue. Several studies have shown that adiponectin can improve glucose and lipid metabolism, insulin sensitivity and atherosclerosis, while lower adiponectin levels are independently associated with a higher risk of CAD [17, 18]. Lin et al. found that adiponectin, as a downstream signal of FGF21, played a significant role in improving the metabolism of glucose and lipid and in enhancing the sensitivity of insulin [19]. Therefore, we speculate that the effect of FGF21 in CAD may be mediated via the activation of adiponectin. However, the exact mechanism between FGF21 and adiponectin still requires further study.

There were some limitations in our study. Firstly, our study population was composed of Chinese middle-and elderly patients presenting at a single hospital. The sample size was relatively inadequate and came from a single center. Therefore, this study demonstrated results for participants who were evaluated for coronary stenosis only by coronary arteriography and not with other coronary evaluation methods. Secondly, opposite results and actions were also reported [15, 20], those studies showed increased level of FGF21 is associated with CAD and extent of the coronary artery. As a novel member of the endocrine FGF subfamily, the mechanisms of FGF21 in the cardiovascular system are still unclear, more multicenter studies should be considered to resolve the controversy problem. In addition, correlation analysis is likely to be affected by a variety of uncorrected confounding factors present in daily life. Furthermore, our finding of the association between serum FGF21 and adiponectin in patients with CAD remains to be confirmed in other population-based studies involving even larger sample sizes and a wider multi-center study.

Conclusions

FGF21 was shown to be one of the independent protective factors for CAD. Serum levels of FGF21 correlated negatively with the severity of coronary artery stenosis, including the Gensini score and the lesion numbers in the coronary artery. Our results suggest that FGF21 has a possible protective effect on patients with coronary heart disease.

Acknowledgements

This work was supported by National Natural Science Foundation of China (81470021) and The Key Clinical Specialty Discipline Construction Program of Fujian, China.

Disclosure of conflict of interest

None.

Address correspondence to: Hai-Feng Chen, Department of Cardiology, Fujian Provincial Clinical College, Fujian Medical University, Fuzhou, Fujian, China. E-mail: drchf@sohu.com

References

[1] Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li D, Mehrbod F, Jaskunas SR, Shanafelt AB. FGF-21 as a novel metabolic regulator. J Clin Invest 2005; 115: 1627-1635.

[2] Planavila A, Redondo-Angulo I, Villarroya F. FGF21 and Cardiac Physiopathology. Front Endocrinol (Lausanne) 2015; 6: 133.

[3] Wang XM, Song SS, Xiao H, Gao P, Li XJ, Si LY. Fibroblast growth factor 21 protects against high glucose induced cellular damage and dysfunction of endothelial nitric-oxide synthase in endothelial cells. Cell Physiol Biochem 2014; 34: 658-671.

[4] Jiang X, Chen J, Zhang C, Zhang Z, Tan Y, Feng W, Skibba M, Xin Y, Cai L. The protective effect of FGF21 on diabetes-induced male germ cell apoptosis is associated with up-regulated testicular AKT and AMPK/Sirt1/PGC-1alpha signaling. Endocrinology 2015; 156: 1156-1170.

[5] Liu SQ, Roberts D, Kharitonenkov A, Zhang B, Hanson SM, Li YC, Zhang L, Wu YH. Endocrine Protection of Ischemic Myocardium by FGF21 from the Liver and Adipose Tissue. Sci Rep 2013; 3: 2767.

[6] Liu SQ, Tefft BJ, Roberts DT, Zhang LQ, Ren Y, Li YC, Huang Y, Zhang D, Phillips HR, Wu YH. Cardioprotective proteins upregulated in the liver in response to experimental myocardial ischemia. AJP: Heart and Circulatory Physiology 2012; 303: H1446-H1458.

[7] Lin Z, Tian H, Lam KS, Lin S, Hoo RC, Konishi M, Itoh N, Wang Y, Bornstein SR, Xu A, Li X. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. Cell Metab 2013; 17: 779-789.

[8] Lin Z, Wu Z, Yin X, Liu Y, Yan X, Lin S, Xiao J, Wang X, Feng W, Li X. Serum Levels of FGF-21 Are Increased in Coronary Heart Disease Patients and Are Independently Associated with Adverse Lipid Profile. PLoS One 2010; 5: e15534.

[9] Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. Am J Cardiol 1983; 51: 606.

[10] Nishimura T, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochim Biophys Acta 2000; 1492: 203-206.

[11] Long YC, Kharitonenkov A. Hormone-like fibroblast growth factors and metabolic regulation. Biochim Biophys Acta 2011; 1812: 791-795.

[12] Lu Y, Liu JH, Zhang LK, DU J, Zeng XJ, Hao G, Huang J, Zhao DH, Wang GZ, Zhang YC. Fibroblast growth factor 21 as a possible endogenous factor inhibits apoptosis in cardiac endothelial cells. Chin Med J (Engl) 2010; 123: 3417-3421.

[13] Shang W, Yu X, Wang H, Chen T, Fang Y, Yang X, Zhou P, Nie F, Zhou Q, Zhou J. Fibroblast growth factor 21 enhances cholesterol efflux in THP-1 macrophage-derived foam cells. Mol Med Rep 2015; 11: 503-508.

[14] Lin Z, Pan X, Wu F, Ye D, Zhang Y, Wang Y, Jin L, Lian Q, Huang Y, Ding H, Triggle C, Wang K, Li X, Xu A. Fibroblast growth factor 21 prevents atherosclerosis by suppression of hepatic sterol regulatory element-binding protein-2 and induction of adiponectin in mice. Circulation 2015; 131: 1861-1871.

[15] Kim WJ, Kim SS, Lee HC, Song SH, Bae MJ, Yi YS, Jeon YK, Kim BH, Kim YK, Kim IJ. Association between Serum Fibroblast Growth Factor 21 and Coronary Artery Disease in Patients with Type 2 Diabetes. J Korean Med Sci 2015; 30: 586-590.

[16] Lee Y, Lim S, Hong E, Kim JH, Moon MK, Chun EJ, Choi SI, Kim Y, Park YJ, Park KS, Jang HC, Choi SH. Serum FGF21 concentration is associated with hypertriglyceridaemia, hyperinsulinaemia and pericardial fat accumulation, independently of obesity, but not with current coronary artery status. Clin Endocrinol 2014; 80: 57-64.

[17] Hascoet S, Elbaz M, Bongard V, Bouisset F, Verdier C, Vindis C, Genoux A, Taraszkiewicz D, Perret B, Galinier M, Carrie D, Ferrieres J, Ruidavets JB. Adiponectin and Long-Term Mortality in Coronary Artery Disease Participants and Controls. Arterioscler Thromb Vasc Biol 2012; 33: e19-e29.

[18] Wang Y, Zheng A, Yan Y, Song F, Kong Q, Qin S, Zhang D. Association between HMW adiponectin, HMW-total adiponectin ratio and early-onset coronary artery disease in Chinese population. Atherosclerosis 2014; 235: 392-397.

[19] Lin Z, Tian H, Lam KS, Lin S, Hoo RC, Konishi M, Itoh N, Wang Y, Bornstein SR, Xu A, Li X. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. Cell Metab 2013; 17: 779-789.

[20] Hanks LJ, Gutiérrez OM, Bamman MM, Ashraf A, Mccormick KL, Casazza K. Circulating levels of fibroblast growth factor-21 increase with age independently of body composition indices among healthy individuals. J Clin Transl Endocrinol 2015; 2: 77-82.