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SERUM LEVELS OF FIBROBLAST GROWTH FACTOR 21 IN TYPE 2 DIABETIC PATIENTS

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Abstract

Background and Purpose. Fibroblast growth factor 21 (FGF21) has recently been identified as a metabolic regulator, but its physiological role is still not completely known. The aim of this study was to evaluate serum FGF21 levels in an Iranian population with type 2 diabetes.

Materials and Methods. This cross-sectional study was conducted in patients with type 2 diabetes. All patients were evaluated for fasting serum levels of glucose, glycated hemoglobin (HbA1c), lipids, urea and creatinine. Participants were divided into two groups with poorly-controlled and well-controlled diabetes based on their HbA1c levels. Healthy non-diabetic subjects (matched with patients in terms of age, sex and body mass index [BMI]) were also recruited as control group. Serum FGF21 concentrations were determined in all subjects using ELISA.

Results. Of the evaluated 141 subjects, 49 (34.8%) were categorized as having well-controlled diabetes, 66 (46.8%) had poorly-controlled diabetes, and there were 26 subjects in the normal control group. Mean serum FGF-21 concentration was 337.89±283.67 ng/L in the diabetic group and 237.25±43.22 ng/mL in the non-diabetic group ($p<0.001$). Mean serum FGF21 level was 237.25 ± 43.22 ng/mL in the control group, 309.81 ± 301.68 ng/mL in the well-controlled diabetic group, and 358.73 ± 269.98 ng/mL in the poorly controlled diabetic group. Serum FGF21 level in the poorly controlled diabetic group was significantly higher than that in the well-controlled diabetic and the healthy control groups ($p=0.02$) but there was no significant difference between the well-controlled and healthy groups. There was no significant association between serum FGF21 levels with lipid levels, presence of diabetic complications and BMI ($p > 0.05$).

Conclusions. The present results suggested an association between elevated serum levels of FGF21 and poor control of diabetes. Future studies are warranted to elucidate the prognostic role of these elevated levels of FGF21 in diabetic subjects.

Key words: Type 2 diabetes, Fibroblast growth factor 21, Insulin resistance, Glycemic control.

INTRODUCTION

Type 2 diabetes mellitus is a major public health burden with a rising global prevalence (1). Fibroblast growth factor 21 (FGF21) is a metabolic factor produced in the liver, adipose tissue, skeletal muscle, and pancreas tissues, and is known to regulate glucose and lipid metabolism (2). It has been recently reported that high serum levels of FGF21 cause abnormal glucose metabolism and insulin resistance in adults (3). Previous animal studies have examined the effects of FGF21 on lipid and glucose metabolism, insulin sensitivity, and body weight (4-6) and suggested of the use of FGF21 as a potential target for the treatment of diabetes and obesity. High serum levels of FGF21 have been reported in patients with obesity, obesity-related diseases, and insulin resistance (7), supporting a potential role for the involvement of this factor in the pathophysiology of cardio metabolic diseases. Owing to the limited number of previous clinical studies, and lack of a previous investigation in the Iranian population, the present study aimed to evaluate the potential association between serum FGF21 concentrations with the presence of diabetes and glycemic control in Iranian subjects with well- and poorly controlled diabetes.

SUBJECTS AND METHODS

Subjects

Patients with type 2 diabetes who were referred to the Imam Reza diabetes clinic in Mashhad from May to July 2014 were selected using convenience sampling approach. Patients were excluded if they had an active infection or inflammation, were taking drugs that affect the immune system (e.g., corticosteroids), had liver or

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kidney disease, or had severe physical inactivity. After a complete explanation of the project, demographic characteristics were recorded, including height, weight, blood pressure, body mass index (BMI), and family history of diabetes. The diagnosis of diabetes was based on the American Diabetes Association criteria (8). A questionnaire was also administered to determine each patient's duration of diabetes, presence of hypertension (determined according to the use of anti-hypertensive drugs or having a blood pressure ≥ 140/90), history of dyslipidemia (lipid abnormalities or use of anti-lipid therapy), presence of cardiovascular disease, and diabetes complications (neuropathy, retinopathy, and nephropathy). The study protocol was approved by the Institutional Ethics Committee.

Blood sampling

Patients, after a 12-hour fast, had 5 mL of their blood drawn from the brachial vein, and all samples were evaluated for FGF21 levels using ELISA technique (Bioassay Technology Laboratory [Crystal Day, China] which gave intra-assay and inter-assay variations of <10% and <12%, respectively. Blood glucose, HbA1c, low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides, cholesterol, urea and creatinine were determined using routine enzymatic techniques. According to HbA1c levels, patients were classified as having well-controlled diabetes (<7%) or poorly controlled diabetes (>7%). Twenty-six healthy subjects who were matched with patients in terms of age, gender and BMI were also recruited as controls.

Statistical analysis

Statistical analyses were performed using SPSS software (version 16). Normal distribution of data was assessed using Kolmogorov–Smirnov test. Variables that had a normal distribution were compared using one-way ANOVA (for continuous variables) and Chi-square (for categorical variables). For non-normally distributed data Kruskal–Wallis and Mann–Whitney U tests, Wilcoxon sign test, and Spearman correlation were used. A p-value of <0.05 was considered significant in this study.

RESULTS

In this study, 141 subjects participated, including 49 patients (34.8%) in the well-controlled diabetes group, 66 patients (46.8%) in the poorly controlled diabetes group, and 26 persons (18.4%) in the healthy non-diabetic control group. These three groups did not have any statistically significant difference in terms of age, gender, BMI, systolic and diastolic blood pressure, triglycerides, and HDL-cholesterol. However, serum glucose, creatinine, and LDL-cholesterol levels were significantly different in at least two groups. Tukey's post-hoc test showed that mean glucose levels were significantly higher in the poorly controlled diabetes patients compared with those with well-controlled diabetes and healthy non-diabetic control subjects, and that patients with well-controlled diabetes group had significantly higher blood glucose compared with the healthy control group as expected. Results from a t-test showed that mean HbA1c levels were significantly greater in patients with poorly controlled diabetes (7.90±1.95%) than in patients with well-controlled diabetes (6.22±0.22%), (p=0.001). Mean serum FGF-21 level was 337.89±283.67 ng/L in the diabetes patients and 237.25±43.22 ng/L in the non-diabetes subjects (p<0.001). The results showed that the mean level of blood creatinine was significantly

Table 1. Comparison of age, body mass index, systolic and diastolic blood pressure, glucose, creatinine and blood lipid profiles in three groups

Group Variable	Well controlled led diabetes mean ± SD	Poorly controlled diabetes mean ± SD	Healthy control mean ± SD	P-value
Age(year)	52.57±11.09	56.15±11.26	51.77±9.78	0.11
Body mass index (kg/m^2)	27.72±3.78	26.98±4.39	26.84±3.76	0.62
Systolic blood pressure (mmHg)	122.56±14.99	123.56±12.25	118.95±9.94	0.40
Diastolic blood pressure (mmHg)	76.67±7.01	78.47±5.51	80.26±3.53	0.08
Blood glucose (mg/dL)	128.10±34.62	196.98±82.81	85.35±11.18	<0.001
HbA1c %	6.22 ± 0.62	7.90±1.95	-	<0.001
Creatinine (mg/dL)	0.91±0.18	1.05±0.19	0.97±0.11	0.001
Triglyceride (mg/dL)	153.71±77.21	161.64±88.87	151.25±81.29	0.83
Cholesterol (mg/dL)	191.22±44.01	189.26±47.02	184.33±34.74	0.93
LDL (mg/dL)	114.84±35.75	113.61±37.78	89.25±29.03	0.01
HDL (mg/dL)	48.98±10.83	45.53±12.49	42.41±6.89	0.06

higher in patients with poorly controlled diabetes than in those with well-controlled diabetes and that mean LDL-cholesterol was significantly lower in the healthy controls than in patients in the well-controlled and poorly controlled diabetes groups (Table 1).

The prevalence of diabetic neuropathy and microalbuminuria was significantly higher in patients with poorly controlled diabetes than in those with well-controlled diabetes. However, the prevalence rates of diabetic retinopathy, cardiovascular disease, hypertension and family history of diabetes were not significantly different in patients in the well-controlled and poorly controlled groups.

Kruskal–Wallis test results showed that, for the mean FGF21 levels in at least two groups, there were significant differences (p=0.002), and the Mann–Whitney U test was used to verify these differences in the both groups. The test results showed that the mean level of FG21 in patients with poorly controlled diabetes was significantly higher than those in patients with well-controlled diabetes (p=0.002) and in the healthy control group (p=0.007). However, the mean FGF21 level did not significantly differ between patients with well-controlled diabetes and participants in the healthy control group (p=0.55) (Table 2).

The mean level of FGF21 was not significantly different among patients with diabetes who had comorbid diabetic retinopathy, neuropathy, heart disease, and microalbuminuria compared to patients with diabetes who did not have these complications (Table 3). In addition, according to Spearman correlation coefficient results did not show any significant relationships between FGF21 level with lipid profiles and creatinine. There was a negative correlation between duration of diabetes with FGF-21 level in well controlled group (p=0.04) but there was no relationship between these variables in poorly controlled diabetes (p=0.44) (Table 4).

Table 2. Comparison of FGF-21 in three groups

Group	No	Mean	Standard deviation
Well controlled diabetes	49	309.81	301.68
Poorly controlled diabetes	66	358.73	269.98
Healthy control	26	237.25	43.22
Total	141	319.33	259.60
P=0.02	=2	df	12.76=x2

DISCUSSION

According to the results of the present research, serum FGF21 concentrations are significantly elevated in people with poorly-controlled diabetes (358.73 ± 269.98) compared with those with well-controlled diabetes, and healthy controls. Another finding was comparable serum levels of FGF21 between the group with well-controlled diabetes and healthy control subjects. The present findings are consistent with those reported by Chavez *et al*. They compared levels of FGF21 in groups of lean, overweight, impaired-fasting glucose and diabetic individuals, and found that diabetic patients' FGF21 levels are higher than other groups. Chavez *et al*. suggested that their findings were related to insulin resistance (9). In another study by Semba *et al*., high serum concentrations of FGF21 were found to be related to abnormal glucose metabolism and insulin resistance in adults (3). In the study by Kralisch *et al*, mean serum concentrations of FGF21 were 2.1 times higher in type 2 diabetic patients (141.8 ng/L) compared with the control group (66.7 ng/L). Non-diabetic patients whose FGF21 levels were undetectable by ELISA also showed a better metabolic

Table 3. Mean comparison of FGF21 in terms of diabetic complications

Diabetic complication	NO		YES		Mann-Whitney U	P
	Mean	Standard deviation	Mean	Standard deviation		
Retinopathy	355.59	305.73	320.72	296.73	753.00	0.15
Neuropathy	306.92	269.65	372.77	328.18	824.50	0.32
Cardiac	341.95	302.44	335.27	302.48	794.50	0.44
Microalbuminuria	337.9	304.53	348.77	272.31	1189.50	0.56

Table 4. Correlation between FGF-21 level with lipid profiles, creatinine and duration of diabetes in the study groups

Variable	Group	TG	CHOL	LDL	HDL	CR	Duration
FGF-21	Well controlled diabetes	r=0.14	r=0.15	r=0.16	r=-0.11	r=-0.04	r=-0.33
		p=0.37	r=0.32	r=0.29	p=0.48	p=0.78	p=0.04
	Poorly controlled diabetes	r=-0.09	r=-0.04	r=-0.10	r=0.13	r=0.10	r=0.11
		p=0.49	p=0.75	p=0.44	p=0.29	p=0.44	p=0.44
	Healthy subjects	r=0.18	r=0.26	r=-0.27	r=-0.10	r=0.22	-
		p=0.39	p=0.62	p=0.21	p=0.63	p=0.29	

profile than those whose FGF21 was measurable (10). According to Mraz *et al*., FGF21 serum levels were significantly higher in patients with comorbid obesity and type 2 diabetes compared with the control group (11). Cheng *et al*. reported that serum FGF21 levels are significantly higher in patients with newly-diagnosed type 2 diabetes and patients who have had type 2 diabetes for 5 years or more compared with healthy subjects. However, the latter study showed no significant difference between serum levels of FGF21 in newly diagnosed type 2 diabetes patients and patients who had type 2 diabetes for longer than 5 years (12).

A study by Li *et al*. determined that fasting plasma levels of FGF21 were significantly higher in patients with new-onset type 2 diabetes and in patients with poorly managed diabetes compared to the control group ($p < 0.05$). They found no significant difference in FGF21 levels when patients with newly-diagnosed and those with poorly controlled diabetes were compared; (13) however, the results of these studies are consistent with our study's findings, which suggest that diabetes is associated with increased serum levels of FGF21.

In this study, there was no significant relationship among levels of triglycerides, cholesterol, HDL, or LDL and FGF21 in any of the three groups. In other studies, a positive association has been found between serum levels of triglycerides and FGF21, and FGF21 has been suggested to play a role in lipid metabolism (14).

Jin *et al.*, in 2014, reported a significant positive association between triglyceride levels and FGF21, however, they found that the association between HDL and FGF21 serum levels was not significant (15). Su *et al*. found that mean HDL in diabetic patients was significantly higher than in healthy individuals (16). In Chavez *et al.*, serum levels of LDL were lower in patients with diabetes than in persons without insulin resistance (9). The results of their studies are not consistent with this study's findings. This inconsistency is probably due to the removal of patients receiving lipid-lowering drugs in the cited studies, whereas in this study, most patients have been treated with drugs that control blood lipids.

The results showed that there was no significant difference between sex, mean age, or BMI and serum levels of FGF21 in any of the three groups. Because the groups were matched for age, sex, and BMI, they did not have any differences in these variables.

In a study by Cheng *et al*., fasting serum FGF21 levels significantly correlated with age and BMI (12). Kralisch *et al*. found that the mean serum levels of FGF21 significantly correlated with BMI and age, but after adjusting for age and BMI, the average concentration of serum FGF21 was significantly lower in women than in men (10).

Jin *et al*. reported a positive correlation among age, BMI, and serum levels of FGF21, but there was no significant relationship between sex and serum FGF21 levels (15).

In this study, no relation was detected between diabetic complications and FGF21 serum levels. There was no significant relationship demonstrated among creatinine or urea levels and FGF21; this result may be due to the fact that the study excluded people with higher than normal creatinine levels. Stein *et al*., however, showed that patients with kidney failure had high levels of FGF21 (17).

Jian *et al*. found an independent association between FGF21 serum levels and urinary albumin excretion in type 2 diabetes patients, indicating that the circulation of FGF21 may be involved in the pathogenesis of diabetic nephropathy (18). An *et al*. showed that FGF21 levels were significantly higher in type 2 diabetes patients with carotid artery plaques than in patients without plaque (19). In another study, serum levels of FGF21 were shown to have higher positive correlations with carotid and iliac lesions in patients with subclinical atherosclerosis than in patients without these complications (20).

While the present study provides the first evidence on the association between serum FGF21 levels with the presence of diabetes and its control status in an Iranian population, a number of limitations deserve to be noted for the present study. The association of serum FGF21 levels with indices of hepatic function was not evaluated in this study. In addition, the cross-sectional nature of this study impedes evaluation of the prognostic value of raised serum FGF21 levels, and the predictive value of these raised levels for future diabetic complications. In conclusion, the results showed that high serum levels of FGF21 were associated with a poor control of blood glucose in diabetic patients. Thus, measurement of serum FGF21 levels might be regarded as a potential indicator for identifying patients with poorly controlled diabetes. However, the role of elevated circulating concentrations of FGF21 in predicting future diabetic, cardiovascular and hepatic events needs to be further explored in future studies.

Conflict of interest

The authors declare that there were no conflicts of interest in this study.

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References

1. International Diabetes Federation (2013) IDF diabetes atlas. 5th ed. Available: http:/www.idf.org/diabetesatlas/5e/the-global-burden/ Accessed 23 April 2013.
2. Woo Y, Xu A, Wang Y, Lam KS. Fibroblast growth factor 21 as an emerging metabolic regulator: clinical perspectives. Clin Endocrinol 2013; 78(4):489-496.
3. Semba RD, Sun K, Egan JM, Crasto C, Carlson OD, Ferrucci L. Relationship of serum fibroblast growth factor 21 with abnormal glucose metabolism and insulin resistance: the Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab 2012; 97(4):1375-1382.
4. Kharitonenkov A1, 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 DS, Mehrbod F, Jaskunas SR, Shanafelt AB. FGF-21 as a novel metabolic regulator. J Clin Invest 2005; 115(6):1627-1635.
5. Dutchak PA, Katafuchi T, Bookout AL, Choi JH, Ruth TY, Mangelsdorf DJ, Kliewer SA. Fibroblast growth factor-21 regulates PPARγ activity and the antidiabetic actions of thiazolidinediones. Cell 2012; 148(3):556-567.
6. Kharitonenkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, Hansen BC, Shanafelt AB, Etgen GJ. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology 2007; 148(2):774-781.
7. Zhang X, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, Wong RL, Chow WS, Tso AW, Lam KS, Xu A. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 2008; 57(5):1246-1253.
8. American Diabetes Association. Standards of medical care in diabetes–2014. Diabetes Care 2014; 37(Suppl 1):S14-80.
9. Chavez AO, Molina-Carrion M, Abdul-Ghani MA, Folli F, DeFronzo RA, Tripathy D. Circulating fibroblast growth factor 21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care 2009; 32(8):1542-1546.
10. Kralisch S, Fasshauer M. Fibroblast growth factor 21: effects on carbohydrate and lipid metabolism in health and disease. Curr Opin Clin Nutr Metab Care 2011; 14(4):354-359.
11. Mraz M, Bartlova M, Lacinova Z, Michalsky D, Kasalicky M, Haluzikova D, Matoulek M, Dostalova I, Humenanska V, Haluzik M.. Serum concentrations and tissue expression of a novel endocrine regulator fibroblast growth factor 21 in patients with type 2 diabetes and obesity. Clinical Endocrinol 2009; 71(3):369-375.
12. Cheng X, Zhu B, Jiang F, Fan H. Serum FGF-21 levels in type 2 diabetic patients. Endocr Res 2011; 36(4):142-148.
13. Li L, Yang G, Ning H, Yang M, Liu H, Chen W. Plasma FGF-21 levels in type 2 diabetic patients with ketosis. Diabetes Res Clin Pract 2008; 82(2):209-213.
14. 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(12):e15534.
15. Jin QR, Bando Y, Miyawaki K, Shikama Y, Kosugi C, Aki N, Funaki M, Noji S. Correlation of fibroblast growth factor 21 serum levels with metabolic parameters in Japanese subjects. J Med Invest 2014; 61(1-2):28-34.
16. Su S, Kuo C, Huang C, Liu C. Fibroblast Growth Factor 21 Predicts Type 2 Diabetes in Taiwanese. The Changhua Journal of Medicine. 2013; 11:42-47.
17. Stein S, Bachmann A, Lössner U, Kratzsch J, Blüher M, Stumvoll M, Fasshauer M. Serum levels of the adipokine FGF21 depend on renal function. Diabetes Care 2009; 32(1):126-128.
18. Jian W-X, Peng W-H, Jin J, Chen X-R, Fang W-J, Wang W-X, Qin l, Dong Y, Su Q. Association between serum fibroblast growth factor 21 and diabetic nephropathy. Metabolism 2012; 61(6):853-859.
19. An S-Y, Lee MS, Yi S-A, Ha ES, Han SJ, Kim HJ, Kim DJ, Lee KW. Serum fibroblast growth factor 21 was elevated in subjects with type 2 diabetes mellitus and was associated with the presence of carotid artery plaques. Diabetes Res Clin Pract 2012; 96(2):196-203.
20. Xiao Y, Liu L, Xu A, Zhou P, Long Z, Tu Y, Chen X, Tang W, Huang G, Zhou Z. Serum fibroblast growth factor 21 levels are related to subclinical atherosclerosis in patients with type 2 diabetes. Cardiovasc Diabetol 2015; 14:72.