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Serum fibroblast growth factor-21 levels and its relationship with carotid intima-media thickness in type 1 diabetes mellitus patients

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ABSTRACT

Objective: The study aimed to evaluate fibroblast growth factor-21 levels in type 1 diabetes patients and its relationship with carotid intima-media thickness which is a marker of atherosclerosis.

Patients and Methods: We recruited 39 patients with type 1 diabetes mellitus and 39 healthy controls. Blood samples for fibroblast growth factor-21, adiponectin and carboxymethyllysine were drawn from subjects after 8 hours fasting. Fasting blood glucose and hemoglobinA1c levels were obtained from patient records. Carotid intima media-thickness was measured via B-mode ultrasound by the same physician.

Results: Median fibroblast growth factor-21 levels were 0.54 (0.10-10.69) ng/ml in type 1 diabetes patients, 0.42 (0.09-1.57) ng/ml in healthy controls (P=0.13). There was no correlation between serum fibroblast growth factor-21 levels and carotid intima-media thickness. Carboxymethyllysine levels were similar in both groups (P=0.86). Adiponectin level was 16336.7 ± 7338.7 ng/ml in type 1 diabetes patients, 13343.1 ± 5318.7 ng/ml in control group (P=0.04).

Conclusion: Our study did not find any relation between serum fibroblast growth factor-21 levels and carotid intima-media thickness. Further researches with wider study population are needed.

Keywords: fibroblast growth factor-21, carotid intima-media thickness, type 1 diabetes mellitus

1. INTRODUCTION

Type 1 diabetes mellitus (DM) is a chronic disease which is characterized by insulin deficiency and usually appears at early ages. Deficiency of insulin leads to hyperglycemia which results in microvascular and macrovascular complications [1].

Fibroblast growth factor-21 (FGF-21) is a regulatory hormone belonging to fibroblast growth factor family. It is synthesized by liver, pancreas and white adipose tissue. FGF-21 level is increased at obesity [2], type 2 DM [3], dyslipidemia [2,3], impaired glucose tolerance [3], nonalcoholic fatty liver disease [4] and coronary artery disease [5]. While serum FGF-21 levels have been shown to increase in type 2 DM, there is limited data in type 1 DM patients. It was demonstrated that FGF-21 levels were decreased in type 1 DM and latent autoimmune diabetes of adult patients [6]. In a study published in 2015, fasting and postprandial FGF-21 levels have been compared between type 1 diabetes and healthy controls. Fasting FGF-21 levels were found to be lower in the diabetic group than controls. In these two studies, the relationship between FGF-21 levels and diabetic complications were not evaluated [6,7].

Type 1 DM is a major risk factor for atherosclerosis. Carotid intima-media thickness (CIMT) which is measured by ultrasonography is initial sign of atherosclerosis and cardiovascular diseases in type 1 diabetes patients [8,9].

Adiponectin synthesized by adipose tissue has an insulin sensitizing effect. While, the level of adiponectin is low in obesity, type 2 DM and coronary artery disease [10], it is increased in type 1 DM [11]. Adiponectin plays an antiatherogenic role by acting directly on endothelial cells and its levels are decreased in coronary artery disease. Determining plasma adiponectin level is considered to be an important parameter in assessing the risk of coronary artery disease [12].

The prolonged exposure to hyperglycemia leads to development of microvascular and macrovascular complications such as nephropathy, neuropathy, retinopathy and atherosclerosis. Advanced glycation end-products (AGE) play a key role in these complications [13]. Carboxymethyllysine is a member of non-crosslinked AGE group and it is a new marker in monitoring the

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development of late complications due to glycation in diabetic patients [14].

Our study aimed to evaluate serum FGF-21 levels in type 1 diabetes patients and to determine its relationship with HbA1c, lipids, adiponectin, AGE (carboxymethyllysine) and carotid intima-media thickness. We hypothesized that FGF-21 levels change in type 1 diabetes mellitus patients and are exhibit positive correlations with CIMT, adiponectin, carboxymethyllysine, LDL, total cholesterol and HbA1c levels.

2. PATIENTS and METHODS

Study Design and Subjects

The study was approved by Marmara University Faculty of Medicine Ethics Committee (Protocol number: 09.2013.0357, date: 20.12.2013). An informed consent was obtained from all study participants.

Type 1 diabetes patients were recruited from outpatient clinics of Endocrinology and Metabolism at Marmara University Hospital. The control group was recruited from individuals applied for general health check-up in Internal edicine outpatient clinics at the same hospital. Type 1 DM patients, having the diagnosis for at least 6 months, aged between 18 and 65 years and having a body mass index (BMI) between 18.5 and 25 kg/cm2, were included. Smoking, chronic inflammatory disease, pregnancy and breastfeeding were the exclusion criteria. Type 1 diabetes patients who have microvascular complications were excluded.

Body mass index was calculated according to weight and height measurements of participants (kg/cm2). Waist circumference was measured. All measurements were obtained by the same physician.

Laboratory Assays

Fasting blood glucose levels, HbA1c, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride and total cholesterol levels which were measured in the last 6 months, have been obtained from the patient's records. After 8 hours fasting, blood samples were obtained to determine the serum FGF-21, adiponectin and carboxymethyllysine levels from all participants. The blood samples were kept at – 80°C refrigerator. All the samples were analyzed together at the end of the study. The FGF-21, carboxymethyllysine and adiponectin kits were based on the sandwich ELISA principle. Kits for FGF-21 and adiponectin were obtained from Assaypro lab (®Assaypro, St Louis, USA). Carboxymethyllysine kits were obtained from Sunred Biotechnology Company (®Sunred Biotechnology Company, Shanghai, China). All steps applied during the test were made according to kit instructions. The intra – and inter-assay variations were 4.7% and 7.2% for FGF-21, 3% and 8.3% for adiponectin, less than 10% and less than 12% for carboxymethillysine, respectively.

Measurement of Carotid Intima-Media Thickness

Carotid intima-media thickness was measured with a B-mode ultrasonography device on the right and left side by the same physician, with the head turned to the opposite side while lying on the supine position. According to the Rotterdam study ultrasonography protocol [9], measurements were made in 3 different regions from the main carotid artery, bifurcation region and proximal internal carotid artery for both sides. Mean values of CIMT measurements were calculated.

Statistical Analysis

Data were analyzed in Statistical Package for Social Sciences (SPSS) 17.0 (Chicago, IL, USA). Continuous variables were defined as mean ± standard deviation and median (minimum-maximum). Variables were compared by independent sample t-test and Mann Whitney-U test. All data were analyzed by Pearson and Spearman correlation analysis. The p value lower than 0.05 was the threshold for the statistical significance.

3. RESULTS

The study included 39 type 1 diabetic patients (13 [33.3%] males, 26 [66.6%] females) and 39 healthy volunteers (12 [30%] males, 27 [70%] females). Both groups were similar regarding gender, age (in patient group 27±8 years, in control group 29 ± 5 years) and BMI (in patient group 22.1 ± 2.1 kg/cm2, in control group 22.7 ± 2.09 kg/cm2).

Variables were compared between type 1 diabetic patients and controls (Table I).

Table I. *Comparison of variables between type 1 diabetes patients and healthy controls*

		Patient (n=39)	Control (n=39)	P
Age		27±8	29 ± 5	0.28
Gender	Female	26	27	0.80
	Male	13	12	
Body Mass Index (kg/cm2)		22.1 ± 2.1	22.7 ± 2.09	0.20
Waist Circumference (cm)		76.4 ± 8.4	74.7 ± 9.1	0.30
CIMT-right side (mm)		0.53 ± 0.05	0.52 ± 0.05	0.30
CIMT-left side (mm)		0.54 ± 0.60	0.51 ± 0.50	0.01
Mean CIMT (mm)		0.54 ± 0.05	0.51 ± 0.05	0.04
Adiponectin (ng/ml)		16336.7 ± 7338.7	13343.1 ± 5318.7	0.04
Fasting Blood Glucose (mg/dl)		193.8 ± 99.8	85.8 ± 9.6	<0.01
Hemoglobin A1c (%)		9.1 ± 2.8	4.9 ± 0.3	<0.01
Total Cholesterol (mg/dl)		180 ± 35	187.6 ± 39	0.37
LDL (mg/dl)		106.5 ± 26.6	114.5 ± 32.5	0.24
HDL (mg/dl)		55 ± 13.9	55 ± 14.3	0.95
Triglyceride (mg/dl)		89.4 ± 42	93.7 ± 60.9	0.71
FGF-21 (ng/ml)		0.54 (0.1-10.6)	0.42 (0.09-1.57)	0.13
Carboxymethyllysine (ng/ml)		577.5 (337.9-2405)	759.3 (170.7-2979.6)	0.86

The data were expressed as mean ± standard deviation or median (minimum-maximum). CIMT: Carotid Intima-Media Thickness, LDL: Low-density Lipoprotein, HDL: High-density Lipoprotein, FGF-21: Fibroblast Growth Factor-21

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There was no correlation between serum FGF-21 levels and other parameters except BMI (Table II). When the diabetic group was separately analyzed, there was a significant correlation between serum FGF-21 levels and BMI (r=0.35, P=0.02). There was no relation between FGF-21 and other variables in the diabetic group.

Serum carboxymethyllysine levels were not correlated with other parameters.

Table II. *Correlation analysis between FGF-21 and other parameters*

	FGF-21	
	r	P
Age	-0.03	0.76
BMI	0.24	0.02
Waist Circumference	0.02	0.85
Mean CIMT	-0.01	0.87
Carboxymethyllysine	0.02	0.84
Adiponectin	0.10	0.34
Fasting Blood Glucose	0.00	0.97
Hemoglobin A1c	0.13	0.22
Total Cholesterol	0.07	0.51
LDL	0.08	0.47
HDL	0.01	0.90
Triglyceride	0.03	0.77

FGF-21: Fibroblast Growth Factor-21, BMI: Body Mass Index, CIMT: Carotid Intima-Media Thickness, LDL: Low-density Lipoprotein, HDL: High-density Lipoprotein.

Adiponectin levels were correlated positively with HbA1c (r =0.2, P=0.01), HDL (r =0.3, P=0.03) negatively with LDL (r =-0.2, P=0.02), triglyceride (P=0.02), waist circumference (r =-0.25, P=0.02) and BMI (r =-0.27, P=0.01).

4. DISCUSSION

Fibroblast growth factor-21 levels were found to be relatively higher in patients with type 1 DM, but this result was not significant. On the other hand, the mean and left-sided CIMT and adiponectin levels were increased in patients with type 1 DM. Carboxymethyllysine levels were found to be similar in both groups. Furthermore, there was no significant correlation between FGF-21 levels and other variables except BMI.

In a study by Xiao et al., association of FGF-21 with different diabetic subtypes has been analyzed and as a result, it was found that FGF-21 levels were increased in type 2 diabetes mellitus, modestly decreased in LADA subtype, and decreased in type 1 DM [6]. Zibar et al., have shown that basal FGF-21 levels of type 1 diabetic patients were lower than controls [7]. Our study found that FGF-21 levels were relatively, but not significantly higher in type 1 diabetic patients compared to the controls. Nevertheless, we cannot conclude that serum FGF-21 levels were increased in type 1 diabetes patients. The age of our study population was higher than age of study population conducted by Xiao et al., and lower than age of study population conducted by Zibar et al. [7]. The difference in serum FGF-21 levels from other studies might have been resulted from age differences in study populations. In a study investigating serum FGF-21 levels and the microvascular complications in type 1 DM showed that FGF-21 levels were decreased in type 1 diabetic patients but there was no relation between its levels and microvascular complications [15]. Association of FGF-21 with macrovascular complications in type 1 diabetic patients has not been evaluated in previous studies [16]. This is the first study to evaluate the association of FGF-21 levels with carotid intima-media thickness which is a marker for atherosclerosis in type 1 diabetics. The measurement of carotid intima-media thickness which is a good indicator of atherosclerosis as a macrovascular complication of diabetes was increased in type 1 diabetic children [17-19]. The results of our study were similar to earlier studies; left-sided and mean CIMT measurements of type 1 diabetic patients were higher than controls but there was no difference between right-sided CIMT measurements. Given the body of existing evidence, we hypothesized that FGF-21 levels might exhibit a positive correlation with CIMT measurements, indicative of early atherosclerosis and a macrovascular diabetic complication [20,21]. However, our study did not find a correlation between CIMT measurements and FGF-21 levels in both the patient and control groups. Unfortunately, we were unable to assess other complications as our patients did not exhibit any additional complications.

In the previous studies, adiponectin levels were increased in patients with type 1 diabetics and patients with retinopathy, nephropathy, cardiovascular complications [22-24]. Our study found that adiponectin levels were increased in type 1 diabetics. However, there was no correlation between adiponectin levels and carotid intima-media thickness. The results of our study confirmed the association of HDL and adiponectin as demonstrated in previous studies [25]. Our study showed that triglyceride, LDL, waist circumference and BMI values decreased as adiponectin levels increased. This data supports the results of previous studies [25,26].

In a study, high serum carboxymethyllysine levels in type 1 diabetic adolescents play a role in diabetic complications and serum carboxymethyllysine levels indicate the long-term control of diabetes [27]. However, in our study, there was no difference in carboxymethyllysine levels between patients with type 1 diabetes and healthy volunteers. Additionally, there was no correlation between carboxymethyllysine and FGF-21, glucose, HbA1c and carotid intima-media thickness.

This is the first and unique study that evaluates association of FGF-21 levels with carotid intima-media thickness in type 1 diabetic patients and controls. It is the strength of our study.

Limitations

There were several limitations in our study, patients with type 1 DM were relatively young and had no known history of diabetic complications; further studies which include type 1 diabetes patients with advanced complications would provide more comprehensive assessment of the association of FGF-21, adiponectin and carboxymethyllysine levels with complications. The primary limitation of our study is the small number of patients in the population. The serum FGF-21 levels may have

shown significant differences if a larger number of patients had been enrolled.

Conclusion

Our study revealed relatively elevated serum FGF-21 levels in individuals with type 1 diabetes, although, the statistical significance was not achieved due to the limited size of our study population. The larger-scale studies are necessary to provide more conclusive results.

Compliance with ethical Standards

Ethics Committee Approval: The study was approved by Marmara University Faculty of Medicine Ethics Committee (Protocol number: 09.2013.0357, date: 20.12.2013). An informed consent was obtained from all study participants.

Conflicts of Interest: The authors declare that they have no conflict of interest relevant to this article.

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