

Serum FGF-21 levels in individuals with prediabetes and newly diagnosed type 2 diabetes mellitus

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ABSTRACT

Aim: To compare serum fibroblast growth factor-21 (FGF-21) levels in healthy individuals, patients with impaired fasting glucose (IFG) and impaired glucose tolerance (IGT); combined prediabetic patients (IFG+IGT), and patients with newly diagnosed type 2 diabetes mellitus (T2DM). In addition, the relationship between serum FGF-21 levels and demographic characteristics, glucose metabolism and laboratory parameters predicting cardiovascular disease risk factors were investigated.

Method: Age, gender, waist and hip circumference measurements, and body mass index (BMI) values were reported for all study groups. Fasting serum insulin, c-peptide levels, insulin/c-peptide ratio, homeostatic model assessment for insulin resistance (HOMA-IR) value, serum lipids, serum cortisol, and plasma fibrinogen levels were all evaluated.

Result: There were no statistically significant variations in the gender distribution of male and female groups ($p = 0.340$). For age, BMI, waist and hip circumference, no statistically significant differences were observed between groups 2, 3, and 4. When the groups were compared for FGF-21 levels, moderate differences were found between Groups 1 and 2 ($p = 0.04$), highly significant differences between Groups 1 and 3 ($p < 0.0001$), and no significant differences were found between Groups 2, 3, and 4 ($p > 0.05$).

Conclusions: Serum FGF-21 levels were significantly increased in prediabetic patients and T2DM. Furthermore, FGF-21 levels were linked to a rise in cardiovascular risk factors. It may shed light on the etiopathogenesis of glucometabolic diseases.

Key words: Fibroblast growth factor-21, impaired fasting glucose, impaired glucose tolerance, prediabetes, type 2 diabetes.

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Introduction

Hyperglycemia that is higher than normal but not high enough to be classified as diabetes mellitus is called prediabetes (PD) [1]. Impaired fasting glucose (IFG), impaired

glucose tolerance (IGT), and combined (IFG+IGT) are the three forms of PD [2]. Early diagnosis and treatment of prediabetic cases are important for avoiding or delaying the onset of type 2 diabetes mellitus (T2DM) [3]. The metabolic biofactor fibroblast growth factor-21 (FGF-21), which is primarily released by the liver and plays a key role in the regulation of carbohydrate and lipid metabolism in the liver, adipose tissue, and pancreas, was recently discovered as a new biomarker [4]. Animal

studies have shown that FGF-21 has potent anti-inflammatory, anti-diabetic, and anti-hyperlipidemic properties [5]. FGF-21 reversed fatty liver, enhanced insulin sensitivity, and increased glucose absorption in adipose tissue in diet-induced obese mice [6,7]. Despite the prominent metabolic function of FGF-21 in laboratory animals, very little information is known about its regulatory effects in humans [8]. Only a few studies have explored the correlation between FGF-21 and glucose metabolism in humans [9]. Based on these results, we intended to look into serum levels of FGF-21, which has been related to a metabolic effect in glucose homeostasis, to better classify those who progress from prediabetic conditions (IFG and combined IFG+IGT) to diabetes mellitus (DM).

The aim was to compare serum FGF-21 levels in healthy individuals, patients with IFG, combined prediabetic patients (IFG + IGT), and newly diagnosed type 2 diabetics. In addition, the relationship between serum FGF-21 levels and demographic characteristics, glucose metabolism and laboratory parameters predicting cardiovascular disease risk factors were investigated.

Materials and methods

Between January and April 2009, 78 patients (51 females and 27 males, ranging in age from 32 to 60 years) who presented to Internal Medicine outpatient clinics were enrolled. Istanbul Taksim Training and Research Hospital Institutional Review Board approved (Date and number: 2009/1730) this single centre, randomized, cross-sectional, prospective research, which was conducted in compliance with the principles of the Helsinki Declaration and classified into four groups: Among the patients who applied to the Internal Medicine outpatient clinic between January and

April 2009, 78 patients (51 females and 27 males, between the ages of 32-60) were randomly selected among those who underwent oral glucose tolerance test OGTT (75 g), and these patients were divided into four groups according to the ADA 2009 (10) diagnostic criteria.

Group 1: Individuals with fasting plasma glucose level <100 mg/dL and 2nd-hour plasma glucose level < 140 mg/dL healthy control control group (n=18);

Group 2: Patients with fasting plasma glucose (FPG) levels of 100 - 126 mg/dL had impaired fasting glucose group (IFG) (n=20);

Group 3: Patients with both IFG and IGT (2nd-hour plasma glucose level 140 - 199 mg/dL, impaired glucose tolerance) combined PD group (IFG + IGT) (n=20);

Group 4: Patients with fasting plasma glucose level \geq 126 mg/dL or 2-hour plasma glucose level \geq 200 mg/dL newly diagnosed T2DM group (n=20).

Exclusion criteria

Patients with one or more disorders such as hyperthyroidism or hypothyroidism, renal and/or hepatic insufficiency, cardiac failure, alcoholism, malignancy, chronic infections, pancreatic diseases, medications. Any factors that could influence pregnancy, puerperium, age of under 18 and metabolic parameters were excluded from the study.

Outcome parameters

Demographic values such as age, gender, waist and hip circumference measurements, and body mass index (BMI) values were registered for all study groups. FPG and in the postprandial 2nd-hour, glycosylated hemoglobin (HbA1c), fasting serum insulin, c-peptide, insulin/c-peptide ratio, homeostatic model assessment for insulin resistance (HOMA-IR) value, serum lipids; total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density

lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglyceride, serum cortisol, and plasma fibrinogen levels were measured.

Venous blood samples from the participants after 10 hours of fasting before OGTT 75 g were taken into K3EDTA tubes for HbA1c and plastic gel serum separation tubes for other tests between 08:00 and 09:00 in the morning. All blood samples were separated from the cell parts by centrifugation at 3000 rpm for 10 minutes.

Except for the FGF-21 test from fasting blood, all other tests were performed on the same day. Serum samples were frozen at -20°C for further study of FGF-21. It was calculated using the formulas $BMI = \text{kg/m}^2$ and $HOMA-IR$ (Homeostasis Model Assessment of Insulin Resistance) = fasting glucose (mg/dL) x fasting insulin ($\mu\text{U/mL}$) / 405.

The serum FGF-21 levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit from Biovendor Research and Diagnostic Products (antibody-coated 96-well plate human FGF-21). The microplate reading system (Biomedical Technologies Inc. USA ELx800) was used to test absorbance at 450 nm (Bio-Tek Instruments, Inc.). Before the study, serum samples were diluted 1:2 by buffer dilution to test FGF-21 levels as directed on the FGF-21 ELISA kit box insert. For the study, the normal curve range was 30-1920 pg/mL. The study had a sensitivity of 7 pg/mL, with intraassay and interassay differences of 3.0-4.1 % and 3.6-3.9 %, respectively.

Statistical analysis

The IBM Statistical Package for Social Sciences v 20 was used to analyze the results (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine that the quantitative data had a

normal distribution. Non-parametric tests (Kruskal-Wallis test and Mann Whitney U test with Bonferroni correction) were used on data with a questionably normal distribution, whereas parametric tests (one-way ANOVA test, posthoc Tukey test, Tamhane's T2 test) were used on data with a normal distribution. The Chi-square test was used to compare predicted and observed values. The correlation coefficients of Pearson (r) and Spearman (rs) were used to assess the relationships between variables. Statistical significance was applied to all variations with a chance likelihood of 0.05 or less.

Results

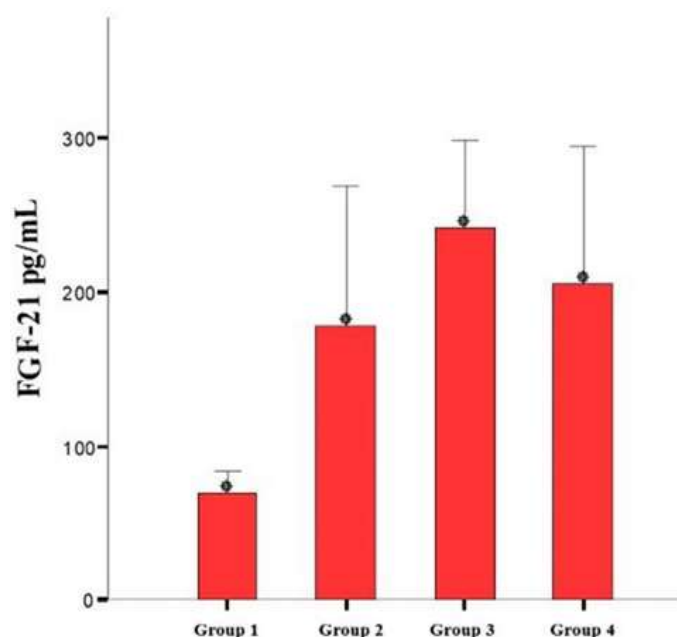
Table 1 shows the distribution of the groups based on demographic characteristics and laboratory results. There were no statistically significant variations in the gender distribution of male and female groups ($p=0.340$). For age, BMI, waist circumference, and hip circumference, no statistically significant differences were observed between groups 2, 3, and 4. Laboratory parameters reflecting glucose metabolism such as FPG, HbA1c, insulin, c-peptide, insulin/c-peptide ratio, HOMA-IR and FGF-21 were statistically significant between groups in the posthoc Tukey analysis ($p < 0.05$ for all). Laboratory parameters reflecting cardiovascular risk factors such as total cholesterol, HDL-C, LDL-C, VLDL-C, TG and fibrinogen were statistically significant between the groups in posthoc Tukey analysis ($p < 0.05$ for all). On the other hand, there was no significant difference between the groups in terms of cortisol levels ($p=0.374$).

Figure 1 shows a comparison of the groups in terms of FGF-21 levels. When the groups were compared for FGF-21 levels, moderate differences were found between Groups 1 and 2 ($p=0.04$), highly significant differences

Table 1. Distribution of groups according to their demographic characteristics and laboratory findings.

| Variable | Group 1 (n=18) | Group 2 (n=20) | Group 3 (n=20) | Group 4 (n=20) | p-value |
|---------------------------|-------------------|--------------------|---------------------|-------------------|----------|
| Age (years) | 42.0 ± 7.8 | 48.9 ± 9.3 | 51.1 ± 7.2 | 48.5 ± 7.0 | 0.006 |
| Gender (M/F) | 8/10 | 9/11 | 5/15 | 5/15 | 0.340 |
| BMI (kg/m ²) | 23.6 ± 3.0 | 30.7 ± 4.9 | 30.4 ± 4.1 | 31.7 ± 2.9 | < 0.0001 |
| Waist circumference (cm) | 85.6 ± 9.4 | 103.5 ± 16.2 | 100.1 ± 10.4 | 101.2 ± 8.9 | < 0.0001 |
| Hip circumference (cm) | 99.5 (93.5-105.0) | 110 (103.5-117.0) | 108 (105.0-116.0) | 110 (104.0-120.0) | 0.004 |
| FPG (mg/dL) | 87.3 ± 7.9 | 108.8 ± 6.1 | 111.9 ± 7.9 | 126.2 ± 14.0 | < 0.0001 |
| Insulin (μU/mL) | 6.6 ± 2.2 | 13.5 ± 6.5 | 14.7 ± 5.6 | 19.5 ± 9.8 | < 0.0001 |
| C-peptide (ng/mL) | 2.0 ± 0.5 | 3.0 ± 1.0 | 3.1 ± 0.9 | 3.6 ± 1.4 | < 0.0001 |
| Insulin/c-peptide | 3.3 ± 0.9 | 4.2 ± 0.8 | 4.5 ± 1.2 | 5.1 ± 1.0 | < 0.0001 |
| HbA1c | 5.4 ± 0.3 | 6.0 ± 0.4 | 6.0 ± 0.4 | 6.5 ± 0.5 | < 0.0001 |
| Total cholesterol (mg/dL) | 174.1 ± 17.9 | 220.0 ± 36.0 | 221.0 ± 37.7 | 201.8 ± 38.4 | < 0.0001 |
| HDL-C (mg/dL) | 54.2 ± 13.1 | 47.4 ± 9.4 | 44.3 ± 13.1 | 42.9 ± 9.1 | 0.016 |
| LDL-C (mg/dL) | 101.5 ± 17.4 | 141.7 ± 31.5 | 135.5 ± 33.8 | 125.0 ± 34.4 | < 0.001 |
| VLDL-C (mg/dL) | 17.0 (11.0-23.0) | 32.0 (19.5- 45.0) | 37.5 (30.0-63.0) | 32.5 (24.5-39.7) | < 0.0001 |
| TG (mg/dL) | 82.5 (56.7-106.7) | 149.5 (89.2-222.2) | 186.5 (150.2-313.2) | 161.5 (123.7-198) | < 0.0001 |
| Cortisol (μg/dL) | 14.2 ± 3.5 | 14.3 ± 6.2 | 15.6 ± 4.0 | 16.7 ± 5.8 | 0.374 |
| HOMA-IR | 1.4 ± 0.5 | 3.6 ± 1.7 | 4.1 ± 1.6 | 6.0 ± 3.1 | < 0.0001 |
| Fibrinogen (mg/dL) | 322.3 ± 57.9 | 450.7 ± 50.5 | 435.6 ± 102.4 | 478.6 ± 129.0 | < 0.0001 |
| FGF-21 (pg/mL) | 69.2 ± 23.8 | 178.2 ± 162.1 | 241.4 ± 103.2 | 205.0 ± 160.9 | < 0.001 |

*Age, BMI, and waist circumference are expressed as $\bar{X} \pm SD$; while waist circumference as median (25-75. percentile). TG and VLDL cholesterol are expressed as median (25. and 75. percentiles), while other parameters as $n\bar{X} \pm SD$. Abbreviations: BMI: Body mass index; FGF-21: Fibroblast growth factor -21, FPG: Fasting plasma glucose; HbA1c: Glycosylated hemoglobin; HDL-C: High-density lipoprotein cholesterol; HOMA-IR: Homeostasis model assessment-insulin resistance; LDL-C: Low-density lipoprotein cholesterol; VLDL-C: Very low-density lipoprotein cholesterol; TG: Triglyceride.

**Figure 1.** Distribution of FGF-21 levels in groups.

between Groups 1 and 3 ($p < 0.0001$), and Groups 1 and 4 ($p = 0.008$), Group 1 was significantly different from group 2, group 3 and group 4 ($p = 0.04$, $p < 0.0001$ and $p = 0.008$, respectively).

The correlation study findings for the groups for FGF-21 were as follows: In group 1, a moderately positive correlation between FGF-21, FBG ($r = 0.493$; $p = 0.038$), TG ($r = 0.489$; $p = 0.040$); a strong positive correlation between FGF-21, c-peptide ($r = 0.528$; $p = 0.024$), VLDL-C ($r = 0.594$; $p = 0.009$) and a weakly negative correlation between FGF-21, and insulin/c-peptide ratio ($r = -0.325$; $p = 0.188$), and a moderately negative correlation between FGF-21, and HDL-C ($r = -0.459$; $p = 0.055$). In group 2, a weakly positive correlation among FGF-21

and LDL-C ($r: 0,363; p = 0,115$), fibrinogen ($r: 0,276; p = 0,239$), HOMA-IR ($r: 0,260; p = 0,267$), waist circumference ($r: 0,309; p = 0,185$); moderately positive correlation between FPG ($r: 0,498; p = 0,025$), total cholesterol ($r: 0,471; p = 0,036$) and TG ($r: 0,444; p = 0,050$). In group 3, a weakly positive correlation between FGF-21 and waist ($r: 0,276; p = 0,240$) and hip ($r: 0,344; p = 0,137$) circumferences; LDL-C ($r: 0,450; p = 0,046$), VLDL-C ($r: 0,441; p = 0,052$), positively strong correlation between total cholesterol ($r: 0,520; p = 0,019$), and TG ($r: 0,448; p = 0,048$). In group 4, a weakly positive correlation between FGF-21, and HbA1c ($r: 0,362; p = 0,117$), VLDL-C ($r: 0,282; p = 0,246$), TG ($r: 0,295; p = 0,250$), and fibrinogen ($r: 0,360$); weakly negative correlation between FGF-21, and the insulin/c-peptide ratio ($r: -0,323; p = 0,165$), cortisol ($r: -0,311; p = 0,181$); a moderately negative correlation between FGF-21, and HDL-C ($r: -0,385; p = 0,093$).

Figure 2 showed a ROC analysis that performed high serum FGF-21 levels can predict the presence of prediabetes ($p < 0.0001$), AUC = 0.830.

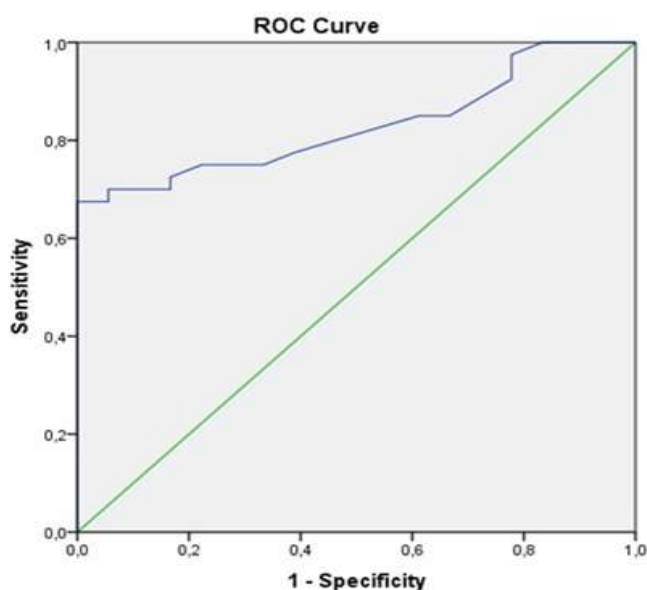


Figure 2. ROC analysis of serum FGF-21 as a predictor of prediabetes.

Discussion

It is important to identify individuals with PD because they have a high risk of developing T2DM (11). The progression from PD to DM takes several years. Various markers, in addition to insulin resistance and insulin secretion defects, can be useful in recognizing people who are at risk of developing diabetes.

PD is not only an intermediate state of dysglycemia but also exists in a continuous cardiometabolic risk between metabolic syndrome (MetS) and T2DM (12).

The research aimed to compare the distribution of FGF-21 serum levels in prediabetic and newly diagnosed T2 DM patients to a healthy control group. From the control to the PD and T2 DM groups, mean values of FPG, insulin, c-peptide, insulin/c-peptide ratio, and HOMA-IR gradually increased.

In one study, FGF-21, triglyceride and LDL-cholesterol levels were significantly higher in type 2 diabetic patients compared to healthy controls; HDL-cholesterol was found to be significantly lower (13).

In our study; FGF-21, LDL-cholesterol and triglyceride were higher in prediabetes and T2 DM groups compared to the control group; had lower HDL-cholesterol levels.

Obesity was the most significant predictor of DM alone, according to Hue et al., who followed 84941 female nurses for 16 years in Nurses Health Study [14]. During follow-up, 3300 of these women without DM were determined to have newly diagnosed DM. Kametani et al. observed the normoglycemic 7222 cases for nine years and found that hypertriglyceridemia was an independent risk factor for IFG and DM development [15]. BMI, body weight, fasting plasma glucose, and plasma triglyceride concentrations were all higher and HDL-C was lower in the prediabetic group than in the healthy control group. The PD

and T2DM groups had higher mean values of BMI, waist, and hip circumference than the control group in our study. When compared to the control group, total cholesterol, triglyceride, LDL-C, and VLDL-C levels increased in the PD and T2DM groups. When compared to the control group, HDL-C levels decreased in the PD and T2DM groups. The Baltimore Longitudinal Study on Aging included 938 prediabetic participants who were followed up for an average of 9.5 years. During follow-up, blood glucose and basal cardiovascular risk factors also increased [16]. Several population-based studies from different ethnic groups have reported that FPG levels of 100 to 109 mg/dL are associated with an increased risk of future diabetes [17].

In our study, there were significant differences in mean FPG levels between the control group, PD groups, and T2DM groups, as well as between T2DM groups and PD groups. The main glucose metabolism parameters used to monitor and control hyperglycemia in T2DM are FPG and HbA1c levels [18]. Our study observed a significant correlation between HbA1c values in the control, PD, and T2DM groups. The correlation between T2DM and IFG was at an advanced level. There were significant differences between the IFG and combined (IFG + IGT) groups. In patients with T2DM, Wakabayashi and Masuda detected a positive significant correlation between plasma fibrinogen levels and atherosclerotic risk factors for cardiovascular disease such as BMI, HbA1c, and total cholesterol, as well as a negative significant correlation between plasma fibrinogen levels and HDL-C [19]. In contrast to the normoglycemic control group, Barillari et al. found a significant increase in plasma fibrinogen levels in T2DM patients [20]. Plasma fibrinogen levels were significantly higher in the PD and T2DM groups than in the

control group in our study. Furthermore, FGF-21 with plasma fibrinogen in T2DM patients, waist, and hip circumference measurements were all positively correlated with BMI, while these measurements were moderately negatively correlated with HDL-C.

Insulin resistance was found to be significantly higher in patients with IGT tests by Tripathy et al. [21]. Liao et al. found that nondiabetic individuals with insulin resistance had an increased risk of developing cardiovascular disease and diabetes [22]. Insulin resistance values calculated using the HOMA-IR test were compared between groups in our study, and these values were extremely higher in PD and T2DM groups compared to the control group, while they differed moderately between T2DM and IFG groups. Insulin resistance is caused by a rise in fasting insulin levels. However, this increase is controlled by the ability of pancreas to secrete insulin, insulin clearance, and glycemia modification. As a consequence, fasting insulinemia is ineffective in detecting insulin sensitivity. The control and IFG groups in our study had significantly different serum fasting insulin levels. There were very significant differences between prediabetes and T2DM groups. Most patients with T2DM have both insulin deficiency and insulin resistance, as is well known. Patients with T2DM did not have low insulin levels in our study as compared to other groups. Studies in adults have suggested the existence of metabolic subphenotypes among prediabetic individuals; while IFG is characterized by a combination of hepatic insulin resistance (IR) and defective early-phase insulin secretion, IGT primarily consists of peripheral IR combined with impaired first-phase and second-phase insulin secretion (23). The c-peptide and/or insulin/c-peptide ratio can also be considered to detect insulin secretion insufficiency. The levels of

insulin, c-peptide, and insulin/c-peptide ratios gradually increased from the control group to the PD and T2DM groups in our study. In our study, significant differences were found between the control group and the PD group, as well as extremely significant differences between the T2DM and control groups, in terms of serum c-peptide levels, which is a good indicator for determining the amount of insulin. FGF-21 may be a promising therapeutic agent for the treatment of DM and other obesity-related metabolic disorders [24]. Another research found that FGF-21 and the anti-diabetic agent rosiglitazone had a strong synergistic effect in stimulating glucose uptake [25]. More significantly, FGF-21 administration resulted in significant improvements in lipoprotein profiles, including lower LDL-C and higher HDL-C, as well as beneficial differences in the levels of some cardiovascular factors in the blood [5]. As a result, FGF-21 remains a promising therapeutic agent for the treatment of DM [7]. Chavez et al. found that plasma FGF-21 levels were significantly higher in the PD and T2DM groups compared to the normoglycemia control group in a study [9]. When compared to the healthy control group, mean FGF-21 values were significantly higher in the PD and T2DM groups in our study. Li et al. compared normoglycemic control, IFG alone, and IGT groups, and found that the IGT group's mean serum FGF-21 concentrations were significantly higher than the control groups [26]. In another study, fasting serum FGF-21 levels were found to increase gradually from control, IGT to T2DM (27). In our study, fasting serum FGF-21 levels were higher than the control groups; IFG had a gradual significant increase up to the (IFG+IGT) combined PD group. Chen et al. found that the newly diagnosed T2DM group had

significantly higher mean FGF-21 levels than the normoglycemic control group [28]. Our study also supports that the T2DM group has significantly higher mean FGF-21 levels than the normoglycemic control group. The other similar parameters include FPG, insulin, HbA1c, HOMA-IR, and TG mean values, all of which were significantly higher in the T2DM group compared to the normoglycemic control group. Li et al. found that mean plasma FGF-21 levels were significantly higher in the newly diagnosed T2DM group than in the normoglycemic control group in a study [29]. Other findings that support our study also include the following: mean FPG, HOMA-IR, HbA1c, and total cholesterol values were significantly higher in the DM group compared to the control group; Mraz et al. found that means fasting serum FGF-21 levels were significantly higher in T2DM group compared to normoglycemic healthy controls [8]. In addition to this finding, the T2DM group had significantly higher mean FPG, BMI, TG, insulin, and HOMA-IR values than the normoglycemic healthy control group.

The relatively limited size of our series was the most important limitation of our study. The second limitation is that the experience is restricted to a single institution's outcomes. Thirdly, certain historical details and variables that may influence the result may not be completely documented. As a consequence of these restrictions, associations should be interpreted with caution.

Conclusions

FGF-21 levels, insulin, c-peptide, insulin/c-peptide ratio, HbA1c, and HOMA-IR parameters all increased from the healthy control group to the development of PD and DM, according to the results of our study. Furthermore, cardiovascular risk factors; metabolic parameters including total

cholesterol, LDL-C, VLDL-C, TG, and fibrinogen all increased in combination with FGF-21 levels. Our study shows that the development of PD and T2DM was correlated to an increase in insulin resistance and serum FGF-21 levels in the healthy normoglycemic control group. Consequently, FGF-21 can be used as a marker of cardiovascular disease in the early detection of glucometabolic abnormalities and insulin resistance.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical statement: Istanbul Taksim Training and Research Hospital Institutional Review Board committee approved the study protocol (Approval ID: 2009/1730).

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