# Fibroblast growth factor-21 is a novel linkage between metabolic parameters, cardiovascular risk, and nephropathy in prediabetes

Sahar H. Elhini[a], Ragaa A. Matta[a], Mahmood Saad[a], Hanan Mostafa[b], Ahmed AbedelfadeeL[b]

[a]Departments ofInternal Medicine, [b]Clinical Pathology, Medical School, Minia University Hospital, Minia, Egypt

Correspondence to Ragaa A. Matta, MD, Department of Internal Medicine, Medical School, Minia University Hospital, Minia, 61111, Egypt; Tel: +20 100 879 2833; fax: 20 86 2156052; e-mail: mattaragaa@yahoo.com

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**Introduction**
Fibroblast growth factor-21 (FGF21) regulates glucose and lipid metabolism and protects against atherosclerosis. Serum FGF21 levels were assessed in newly diagnosed, drug-naive patients with prediabetes (group 1, *n*=60) and diabetes mellitus type 2 (group 2, *n*=60), in addition to 40 healthy individuals (group 3, *n*=40).
**Results**
Serum FGF21 levels were significantly increased in groups 1 and 2 compared with group 3 (231.7±59.3 and 260.4±82.5 vs. 22.6±5.31 pg/dl, respectively; P<0.001 for both). Moreover, group 2 had statistically significantly higher FGF21 levels compared with group 1 (P=0.03). Receiver operating characteristic curve analysis identified FGF21 cutoff value of greater than 204 and 30 pg/ml for the diagnosis of diabetes mellitus type 2 and prediabetes, with an area under the curve 0.72 and 1, sensitivity of 82.5 and 100%, and specificity of 60 and 100%, respectively. Using univariate analysis, FGF21 was positively correlated with blood pressure, obesity (BMI and waist–hip ratio), glycemic (glucose and glycosylated hemoglobin) and insulin resistance (fasting insulin and homeostasis model assessment-insulin resistance) parameters, atherogenic lipid profile, liver enzymes, and cardiovascular disease risk score in group 1 and group 2. FGF21 correlated with albumin–creatinine ratio negatively in group 1 and positively in group 2. Independent predictors of FGF21 level were fasting glucose, insulin, and triglyceride in both patient groups. The independent predictors of FGF21 were obesity parameters in group 1 and albumin–creatinine ratio, age, and systolic blood pressure in group 2.
**Conclusion**
Among prediabetic patients, FGF21 is an excellent predictor and a novel linkage between metabolic parameters, circulatory system, and nephropathy.

**Keywords:**
albumin-to-creatinine ratio, cardiovascular disease risk score, fibroblast growth factor-21, metabolic syndrome parameters, prediabetes

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## Introduction

Prediabetes can be identified as either impaired fasting glucose or impaired glucose tolerance (IGT). Both are risk factors for diabetes mellitus type 2 (DMT2) and cardiovascular disease (CVD). Prediabetes is caused primarily by a deficiency in insulin secreted by pancreatic beta cells and insulin resistance (IR). It commonly associates with the metabolic syndrome and carries some predictive power for macrovascular but has only a minor impact on microvascular complications [1].

Fibroblast growth factor-21 (FGF21) is a novel metabolic hormone and belongs to FGF superfamily. It is expressed in the liver predominantly, and in adipose tissue, skeletal muscle, and pancreas. FGF21 possesses potent beneficial effects on glucose and lipid metabolism and enhances insulin sensitivity. It binds to its receptors and its coreceptor β-Klotho, a single transmembrane protein in these metabolic tissues in endocrine and autocrine manners [2,3].

An increased concentration of FGF21 is closely associated with many cardiometabolic disorders – namely, metabolic syndrome, obesity, DMT2, dyslipidemia, nonalcoholic fatty liver diseases (NAFLD), and coronary artery disease [4]. Moreover, in two 5-year follow-up studies, high levels of FGF21 predicted impaired glucose metabolism and DMT2 [5,6]. Recent studies showed associations of serum FGF21 with macrovascular and microvascular complications of DMT2 [7]. In 2-year

and 5-year follow-up studies, high baseline FGF21 was associated with a higher risk for cardiovascular events, morbidity, and mortality in diabetic patients [8,9]. We hypothesized that FGF21 may be correlated to CVD risk score. Cardiovascular risk assessment is based on the combination of predictive information from several cardiovascular risk factors using mathematical equations. D'Agostino–Framingham Risk Score is the estimation of 10-year CVD risk of a person. The ADVANCE risk score, which was recently developed, showed acceptable performance for predicted 4-year risk for major CVD among patients with DMT2 [10,11]. However, there were conflicting data on elevated FGF21 levels in diabetic and prediabetic patients [12,13]. In many of these studies, diabetic patients were under DM therapy and peroxisome proliferator-activated receptor agonist that affect circulating FGF21 levels [14–16]. We investigated serum FGF21 levels in newly diagnosed, drug-naive patients with prediabetes and DMT2 and explored its relationships with anthropometric, metabolic, IR, hepatic, and renal parameters, and CVD risk score. We also aimed to assess predictors of FGF21 level in these patients, particularly in prediabetic patients, an aspect sparsely mentioned before.

## Patients and methods

This prospective cross-sectional study was conducted on 120 newly diagnosed, drug-naive patients with DMT2 (n=60) and prediabetes (n=60), in addition to 40 age-matched and sex-matched healthy individuals who served as the control group. The patients were selected from attendants of the diabetic outpatient clinic at the Internal Medicine Department, Minia University Hospital, along the period from March 2014 to April 2016.

### Ethical aspects

The study protocol was approved by the Institutional Ethics Committee and conducted in accordance with the ethical guidelines of the Declaration of Helsinki. All patients provided informed consent to participate in this study.

This study involved three groups: the prediabetic, diabetic, and healthy control groups. Participants' ages ranged from 35 to 60 years with a mean±SD of 47.6±7.2 years in the prediabetic group [male/female (m/f): 26/34], from 37 to 60 years with a mean±SD of 48.4±6.2 years in the diabetic group (m/f: 24/36), and from 36 to 60 years with a mean±SD of 47.2±6.9 years in the healthy control group (m/f: 16/24). The criteria of The American Diabetes Association's Standards of Medical Care (2014) were used for the diagnosis of prediabetes and DMT2. Prediabetes is the term used for individuals with impaired fasting glucose as follows: fasting plasma glucose (FPG) of 100–125 mg/dl; IGT as 2 h plasma glucose (PG) in the 75 g oral glucose tolerance test (OGTT) of 140–199 mg/dl; or glycosylated hemoglobin (HbA1c) of 5.7–6.4%. DM was diagnosed as either FPG of 126 mg/dl or more, 2 h PG of 200 mg/dl or more during an OGTT, and HbA1c up to 6.5% or classic symptoms of hyperglycemia or hyperglycemic crisis and a random PG of 200 mg/dl or more [17].

All participants answered a standardized questionnaire including age, conventional CVD risk factors, and current medication. Arterial blood pressure (BP) was measured after 15 min of rest. Systemic arterial hypertension (HTN) was defined as BP 140/90 or more according to the European Society of Hypertension and the European Society of Cardiology Guidelines 2013 [18]. Anthropometric measurements were taken in a standardized manner; BMI was calculated by dividing body weight (kg) by square of height ($m^2$). Waist and hip circumferences were measured and waist–hip ratio (WHR) was calculated according to WHO [19].

### Exclusion criteria

Patients with overt hepatic, renal, and CVDs, malignancy, and chronic inflammatory disease, or those taking any antihyperglycemic medications, insulin sensitizers, steroid, statin, or fibrates were excluded.

### Laboratory investigation

Blood samples were drawn at 08:00 a.m. after overnight fasting at the time of OGTT and after 12 h overnight fast for lipid profile on another day. All samples were collected and processed for hormonal and biochemical assay according to standard biochemistry assay. Plasma and serum samples were subsequently stored in aliquots at −80C° until further analysis of fasting insulin and serum FGF21.

Biochemical assays including PG, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C), triglycerides (TGs), creatinine, urea, liver albumin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using standard laboratory methods. The value (%) of HbA1c was measured and corrected to the value defined by the National Glycohemoglobin Standardized Program. Fasting plasma insulin was measured using immunoenzymometric assay. The homeostasis model assessment (HOMA-IR) index was calculated as previously described using the following formula: fasting plasma insulin

(μIU/ml)×FPG (mg/dl)/405 [20]. Estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft–Gault formula: creatinine clearance= [(140−age)×weight)/(serum creatinine×72)]×0.85 in female patients [21].

Serum FGF21 levels were measured by means of ELISA (FGF21 Quantikine Human ELISA kit, R&D Systems, Minneapolis, Minnesota, USA) following the manufacturer's instructions. The standard curve range for the assay was 31.3–2000 pg/ml. Each sample was assayed in duplicate, and the mean value of the two measures was used for the analyses.

Urine samples were collected for the calculation of albumin-to-creatinine ratio (ACR) to define the grades of albuminuria according to American Diabetes Association criteria 2005 [22].

Global cardiovascular risk score was calculated using D'Agostino–Framingham risk score among prediabetic patients and using ADVANCE score among diabetic patients. D'Agostino–Framingham risk score is a sex-specific multivariable risk factor algorithm and can be conveniently used to assess general CVD risk factors, including age, total and HDL-C, systolic blood pressure (SBP), treatment for HTN, smoking, and diabetes status [10]. ADVANCE model equation included and pointed ten cardiovascular risk factors. They were age at diagnosis and duration of DM, sex, atrial fibrillation, retinopathy, treated HTN, pulse pressure, HbA1c (%), albuminuria, and non-HDL-C. ADVANCE model equation excellently predicts 4-year CVD risk [11,23].

Diagnostic criteria for NAFLD: it was diagnosed using B ultrasonography. Hepatic steatosis was defined as a diffuse increase in fine echoes in the liver parenchyma compared with that in the kidney or spleen parenchyma according to the Prevention and Treatment Guidelines for NAFLD 2010 published by the Society of Hepatology, Chinese Medical Association [24].

### Statistical analysis

All statistical analyses were performed with the statistical package for the social sciences, version 20 (SPSS; SPSS Inc., Chicago, Illinois, USA). Quantitative data are expressed as mean and SD, and as frequencies for categorical variables. Differences between groups were assessed using analysis of variance, Student's unpaired t-test, Mann–Whitney U-test, or $\chi^2$-test, as appropriate. Correlation analysis was performed using the Pearson correlation method. To identify independent relationships and adjust the effects of covariates, stepwise multiple linear regression analyses were performed including all parameters with highly significant correlations in the univariate analysis ($P<0.01$) as covariates. In case of parameters strongly related to each other, one representative covariate was included in the model. Receiver operating characteristic (ROC) curve analysis was performed to assess cutoff point of TGF21 for the diagnosis of prediabetes and DM. P less than 0.05 was considered statistically significant.

## Results

Baseline characteristics of prediabetic, diabetic, and healthy participants are summarized in Table 1. Serum FGF21 levels were significantly increased in prediabetic and diabetic participants compared with healthy controls (231.7±59.3 and 260.4±82.5 vs. 22.6 ±5.31 pg/dl, respectively, $P<0.001$ for both). Moreover, diabetic patients had statistically significantly higher FGF21 levels compared with prediabetic patients ($P=0.03$). ROC curve analyses identified FGF21 levels greater than 204 pg/dl as cutoff value for the diagnosis of DMT2 with area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of 0.72, 82.5, 60, 67.3, 77.4, and 71.25%, respectively (Fig. 1). ROC curve analyses identified FGF21 cutoff value greater than 30 pg/dl for the diagnosis of prediabetes with AUC of 1 and 100% for sensitivity, specificity, NPV, PPV, and accuracy (Fig. 2).

Univariate correlations among all participants: serum FGF21 levels were positively associated with age, SBP and diastolic BP, parameters of obesity (BMI, waist circumference, and WHR), parameters of insulin and glucose metabolism [fasting glucose, 2-h postprandial (pp) blood glucose, HbA1c, fasting insulin, and HOMA-IR], dyslipidemia (TC, LDL-C, and TG), and liver enzymes (ALT and AST) and ACR. Moreover, serum FGF21 levels were negatively correlated with HDL-C and eGFR ($P<0.001$ for all; $P=0.003$ for ALT). In contrast, no association of FGF21 with blood urea and serum creatinine was demonstrated.

Univariate correlations in prediabetic patients: in the prediabetic group, serum FGF21 level had a similar correlation as in all participant groups, with the exception of the presence of a negative correlation with ACR ($P=0.02$) and absence of correlation with eGFR. It had a positive correlation with age, SBP and diastolic BP ($P=0.02$, 0.02, and 0.03, respectively), obesity parameters (BMI, waist circumference, and WHR; $P=0.005$, 0.006, and 0.002, respectively),

**Table 1 Baseline characteristics of studied groups**

| Variables | Groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Group I (prediabetic group) (*N*=60) | Group II (diabetic group) (*N*=60) | Group III (control group) (*N*=40) | A | B | C | D |
| Fibroblast growth factor-21 (pg/dl) | | | | <0.001* | 0.03* | <0.001* | <0.001* |
| Range | 145–435 | 179–439 | 12–30 | | | | |
| Mean±SD | 231.7±59.3 | 260.4±82.5 | 22.6±5.31 | | | | |
| Age (years) | | | | 0.78 | 0.67 | 0.9 | 0.59 |
| Range | (35–60) | (37–60) | (36–60) | | | | |
| Mean±SD | 47.6±7.2 | 48.4±6.2 | 47.2±6.9 | | | | |
| Sex [*n* (%)] | | | | 0.95 | 0–83 | 0.83 | 1 |
| Male | 26 (43.3) | 24 (40) | 16 (40) | | | | |
| Female | 34 (56.7) | 36 (60) | 24 (60) | | | | |
| Waist circumference (cm) | | | | <0.001* | 0.003* | <0.001* | 0.003* |
| Range | (84–110) | (79–112) | (68–92) | | | | |
| Mean±SD | 95.3±8.2 | 90.2±10.2 | 79.1±7.87 | | | | |
| Waist/hip ratio | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (0.83–0.99) | (0.76–0.99) | (0.7–0.87) | | | | |
| Mean±SD | 0.9±0.05 | 0.85±0.07 | 0.8±0.06 | | | | |
| BMI (kg/m$^2$) | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (30.6–51.5) | (24–56) | (22.2–27) | | | | |
| Mean±SD | 35.5±3.8 | 32.03±3.4 | 24.8±1.25 | | | | |
| Systolic blood pressure (mmHg) | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (130–210) | (110–190) | (100–120) | | | | |
| Mean±SD | 146.3±15 | 158±16.6 | 113±6.48 | | | | |
| Diastolic blood pressure (mmHg) | | | | <0.001* | 0.19 | <0.001* | <0.001* |
| Range | (80–110) | (70–110) | (70–80) | | | | |
| Mean±SD | 94.2±7.1 | 96.2±9.6 | 74±4.96 | | | | |
| Fasting plasma glucose (mg/dl) | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (101–125) | (155–299) | (73–91) | | | | |
| Mean±SD | 110.3±6.8 | 204.7±33.7 | 82.9±6.57 | | | | |
| 2-h postprandial plasma glucose (mg/dl) | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (141–194) | (221–492) | (99–120) | | | | |
| Mean±SD | 162.5±16.2 | 344.3±99.5 | 109±5.7 | | | | |
| Hemoglobin A1c (%) | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (5.7–6.4) | (6.9–15) | (4–5.2) | | | | |
| Mean±SD | 6.01±0.24 | 9.61±2.33 | 4.65±0.46 | | | | |
| Fasting insulin (μU/ml) | | | | <0.001* | 0.09 | <0.001* | <0.001* |
| Range | (9.61–34) | (10.57–30) | (4.29–8.74) | | | | |
| Mean±SD | 19.8±8.5 | 21.7±5.6 | 6.36±1.68 | | | | |
| HOMA-IR | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (0.6–9.1) | (3.46–30) | (0.7–1.7) | | | | |
| Mean±SD | 5.4±2.0 | 11.3±4.3 | 1.17±0.34 | | | | |
| Total cholesterol (mg/dl) | | | | <0.001* | 0.007* | <0.001* | <0.001* |
| Range | (119–286) | (127–291) | (100–187) | | | | |
| Mean±SD | 186.4±45.7 | 209.7±47.21 | 151±32 | | | | |
| Triglycerides (mg/dl) | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (60–308) | (54.3–410) | (39–138) | | | | |
| Mean±SD | 148.2±50.5 | 237.1±114 | 79±33 | | | | |
| High-density lipoprotein (mg/dl) | | | | <0.001* | 0.1 | <0.001* | <0.001* |
| Range | (35–62) | (35.7–67) | (60–72) | | | | |
| Mean±SD | 48.3±10.3 | 45.6±7.6 | 66±3.92 | | | | |
| Low-density lipoprotein (mg/dl) | | | | <0.001* | 0.49 | <0.001* | <0.001* |
| Range | (44–210) | (50–212.2) | (26–69) | | | | |
| Mean±SD | 109.1±46.8 | 114.8±43.3 | 41±21 | | | | |
| Serum creatnine (mg/dl) | | | | 0.01* | 0.76 | 0.001* | 0.01* |
| Range | (0.4–1.3) | (0.5–1.3) | (0.7–0.8) | | | | |

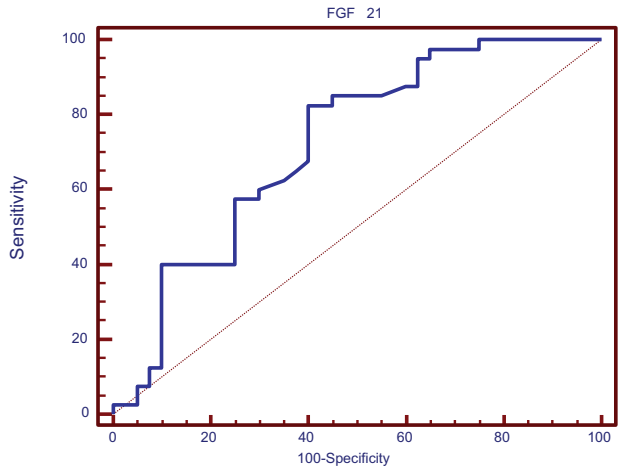
(*Continued*)

**Table 1** (***Continued***)

| Variables | Groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Group I (prediabetic group) (*N*=60) | Group II (diabetic group) (*N*=60) | Group III (control group) (*N*=40) | A | B | C | D |
| Mean±SD | 0.87±0.2 | 0.85±0.3 | 0.74±0.03 | | | | |
| Blood urea (mg/dl) | | | | 0.09 | 0.98 | 0.030* | 0.03* |
| Range | (20–60) | (18–68) | (26–39) | | | | |
| Mean±SD | 40±11.58 | 43.3±11.91 | 32.2±4.91 | | | | |
| Albumin creatnine ratio | | | | <0.001* | <0.001* | 0.002* | <0.001* |
| Range | (15–30) | (30–100) | (10–30) | | | | |
| Mean±SD | 22.3±4.2 | 54.2±19.5 | 19.7±5.9 | | | | |
| eGFR (ml/min) | | | | <0.001* | 0.67 | <0.001* | <0.001* |
| Range | (100–190) | (67–187) | (126–168) | | | | |
| Mean±SD | 128.2±31.5 | 117.9±36.6 | 149±12.9 | | | | |
| Kidney echogenicity (ultrasongraphy) [*n* (%)] | 5 (8.3) | 24 (40) | 0 (0) | <0.001* | <0.001* | <0.001* | <0.001 |
| Alanine aminotransferase (U/l) | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (10–62) | (15–67) | (14–46) | | | | |
| Mean±SD | 36.6±15.3 | 46.6±17.3 | 24.4±9.89 | | | | |
| Aspartate aminotransferase (U/l) | | | | <0.001* | <0.001* | <0.001* | <0.001* |
| Range | (14–70) | (15–72) | (11–30) | | | | |
| Mean±SD | 36.5±15.2 | 43.6±14.3 | 19.7±6.51 | | | | |
| Fatty liver changes (ultrasongraphy) [*n* (%)] | 45 (75) | 44 (73.3) | 0 (0) | <0.001* | 0.79 | <0.001* | <0.001* |
| Hepatomegaly (ultrasongraphy) [*n* (%)] | 40 (66.7) | 24 (40) | 0 (0) | <0.001* | 0.04* | <0.001* | <0.001* |

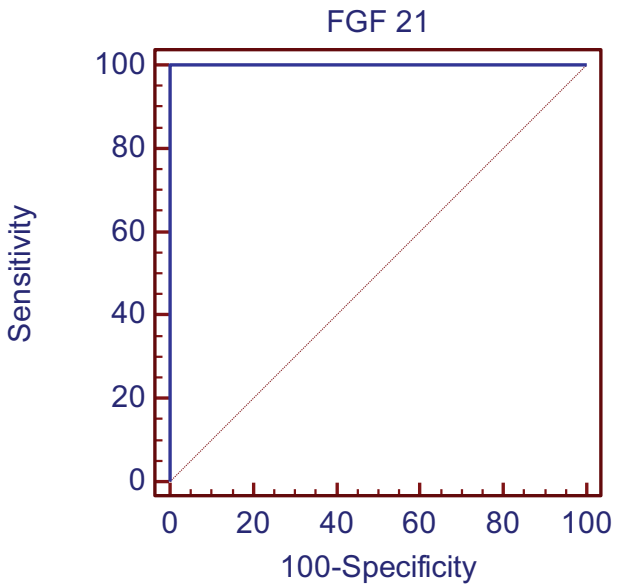
Quantitative data are expressed as mean±SD and compared using the analysis of variance test followed by post-hoc Tukey correction, whereas qualitative variables are expressed as frequency and compared by $\chi^2$-test. eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model assessment of insulin resistance. A: *P* value between the three groups. B: *P* value when group I versus group II. C: *P* value when group I compared with group III. D: *P* value when group II compared with group III. *$P<0.05$, significant.

**Figure 1**




Receiver operating characteristic curve analyses identified fibroblast growth factor-21 (FGF21) levels of 204 pg/dl as cutoff value for the diagnosis of diabetes mellitus type 2 with 0.72 for area under the curve, 82.5% sensitivity, 60% specificity, 67.3% positive predictive value, 77.4% negative predictive value, and 71.25% accuracy

**Figure 2**




Receiver operating characteristic curve analyses identified fibroblast growth factor-21 (FGF21) levels of 30 pg/dl as cutoff value for the diagnosis of prediabetes with 1 for AUC and 100% for sensitivity, specificity, NPV, PPV, and accuracy

and glycemic and IR parameters [fasting glucose, fasting insulin, and HOMA-IR (<0.001 for all); 2-hpp blood glucose and HbA1c (P=0.001 for both)]. It was positively correlated with TG, TC, and LDL-C (P<0.001, P=0.002, and P=0.01, respectively) and negatively correlated with HDL-C (P=0.007). It was positively correlated with liver enzymes (0.01 for both) (Table 2).

**Table 2 Correlation between serum fibroblast growth factor-21 with clinical and biochemical parameters and cardiovascular disease risk score among all patients and studied patient groups**

| | All participants (*n*=160) | | Prediabetic group (*n*=60) | | Diabetic group (*n*=60) | |
|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* |
| Age (years) | 0.5 | <0.001* | 0.33 | 0.02* | 0.66 | <0.001* |
| Waist circumference (cm) | 0.54 | <0.001* | 0.35 | 0.006* | 0.37 | 0.004* |
| Waist/hip ratio | 0.40 | <0.001* | 0.39 | 0.002* | 0.42 | 0.001* |
| BMI (kg/m$^2$) | 0.70 | <0.001* | 0.36 | 0.005* | 0.51 | <0.001* |
| Systolic blood pressure (mmHg) | 00.63 | <0.001* | 0.3 | 0.02* | 0.64 | <0.001* |
| Diastolic blood pressure (mmHg) | 00.71 | <0.001* | 0.27 | 0.03* | 0.57 | <0.001* |
| Fasting plasma glucose (mg/dl) | 00.59 | <0.001* | 0.45 | <0.001* | 0.72 | <0.001* |
| 2-h postprandial plasma glucose (mg/dl) | 00.56 | <0.001* | 0.41 | 0.001* | 0.33 | 0.01* |
| Hemoglobin A1c (%) | 00.52 | <0.001* | 0.44 | 0.001* | 0.49 | <0.001* |
| Fasting insulin (μU/ml) | 0.70 | <0.001* | 0.47 | <0.001* | 0.37 | 0.003* |
| HOMA-IR | 0.559 | <0.001* | 0.45 | <0.001* | 0.62 | <0.001* |
| Total cholesterol (mg/dl) | 0.395 | <0.001* | 0.38 | 0.002* | 0.39 | 0.002* |
| Triglycerides (mg/dl) | 0.43 | <0.001* | 0.46 | <0.001 | 0.65 | <0.001* |
| High-density lipoprotein (mg/dl) | –0.666 | <0.001* | –0.34 | 0.007* | –0.43 | 0.001* |
| Low-density lipoprotein (mg/dl) | 0.58 | <0.001* | 0.21 | 0.01* | 0.12 | 0.44 |
| Serum creatnine (mg/dl) | 0.12 | 0.11 | 0.21 | 0.11 | –0.16 | 0.22 |
| Blood urea (mg/dl) | 0.13 | 0.09 | 0.22 | 0.08 | 0.22 | 0.08 |
| Albumin–creatinine ratio | 0.53 | <0.001* | –0.28 | 0.02* | 0.48 | <0.001* |
| eGFR (ml/min) | –0.28 | <0.001* | –0.16 | 0.22 | 0.17 | 0.18 |
| Alanine aminotransferase (U/l) | 0.273 | 0.003* | 0.31 | 0.01* | 0.45 | <0.001* |
| Aspartate aminotransferase (U/l) | 0.394 | <0.001* | 0.31 | 0.01* | 0.43 | <0.001* |

eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model assessment of insulin resistance. Correlation using Pearson's coefficient. *$P$<0.05, significant.

Univariate correlations were performed in diabetic patients: in the diabetic group, serum FGF21 levels had similar correlations as in all groups except for LDL-C and eGFR, with P less than 0.001 for age, SBP and diastolic BP, BMI, fasting glucose, HbA1c, HOMA-IR, TG, liver enzymes, and ARC; the probability values were as follows: P=0.004, 0.001, 0.01, 0.003, 0.002, and 0.001 for waist circumference, WHR, 2-hpp blood glucose, fasting insulin, TC, and HDL-C, respectively (Table 2).

Moreover, FGF21 was significantly positively correlated to cardiovascular risk score assessed using Framingham's risk score in prediabetic patients (r=0.35, P=0.006) and assessed using ADVANCE score in diabetic patients (r=0.83, P<0.001) (data are not shown).

Factors that were highly significantly correlated to FGF21 were selected for multiple stepwise linear regression analysis. In the prediabetic group, BMI, WHR, fasting glucose, 2-hpp blood glucose, HbA1c, fasting insulin, HOMA-IR, cholesterol, TG, and HDL-C were selected for multiple stepwise linear regression analysis as independent variables and FGF21 as dependent variable. Fasting insulin, TG, BMI, fasting glucose (P<0.001 for all), and WHR (P=0.003) were significant contributors to FGF21 level in the prediabetic group (Table 3).

In the diabetic group, age, SBP and diastolic BP, BMI, WHR, fasting glucose, HbA1c fasting insulin, HOMA-IR, TC, TG, ACR, ALT, and AST were selected for multiple stepwise linear regression analysis as independent variables and FGF21 as dependent variable. TG, ACR (P<0.001 for both), age, fasting glucose (P=0.001 for both), SBP (0.006), and fasting insulin (0.04) were significant contributors to FGF21 level in the diabetic group.

## Discussion

In the current study, we found significantly elevated serum FGF21 levels among newly diagnosed treatment-naive patients with either prediabetes or DMT2 compared with healthy controls. Moreover, FGF21 level progressively increased from prediabetic to overt DMT2 with novel identification of cutoff value greater than 30 and 204 for their diagnosis, respectively, an issue rarely explored before. We were pioneering to identify the association of FGF21 with cardiometabolic risk factors, IR indices, hepatic enzymes, ACR, and CVD risk score among prediabetic patients.

FGF21 functions as a metabolic regulator in either endocrine or autocrine manner in multiple organs, including blood vessels, liver, adipose tissue, kidney, heart, skeletal muscle, and brain. FGF21 acts on the

**Table 3 Stepwise multiple linear regression analysis with fibroblast growth factor-21 as dependent variable among prediabetic and diabetic patients**

| | Independent variables in prediabetes | | Independent variables in diabetes | |
|---|---|---|---|---|
| | *β* | *P* | *β* | *P* |
| Age (years) | – | – | 0.25 | 0.001* |
| Systolic blood pressure (mmHg) | – | – | 0.19 | 0.006* |
| BMI (kg/m$^2$) | 0.37 | <0.001* | – | – |
| Waist–hip ratio | 0.43 | 0.003* | – | – |
| Fasting plasma glucose (mg/dl) | 0.10 | <0.001* | 0.26 | 0.001* |
| Fasting insulin (μU/ml) | 0.24 | <0.001* | 0.12 | 0.04* |
| Triglyceride (mg/dl) | 0.18 | <0.001* | 0.30 | <0.001* |
| Albumin–creatinine ratio | – | – | 0.25 | <0.001* |

*Significant correlation.

above organs not only by directly binding to its receptors of these organs in the presence of β-Klotho but is also mediated by adiponectin or the central neural system [14].

Similar to our results, a previous Chinese cohort study reported elevated FGF21 levels among diabetic and prediabetic patients [5]. However, these patients were under medications (as antihyperglycemic, fibrate, statin, metformin, and insulin sensitizers), which may affect FGF21 levels [14–16]. Many studies in newly diagnosed diabetic patients and only one small study carried out on treatment-naive (12 prediabetic and 12 diabetic patients) reported similar results [25–27]. In contrast, others reported no significant difference of FGF21 between DMT2 and healthy controls [12,13]. Our study provided this novel additive value in daily clinical practice to help clinician obtain a reference for the diagnosis of diabetes and prediabetes in Egyptian population. We demonstrated FGF21 levels of 204 pg/dl as cutoff value for the diagnosis of DMT2 with AUC, sensitivity, specificity, PPV, NPV, and accuracy of 0.72, 82.5, 60, 67.3, 77.4, and 71.25%, respectively. Comparable results were found for T2DM. The best cutoff value for the prediction of diabetes was greater than 322.9 pg/l with an AUC of 0.723 in Taiwanese participants [28]. We were pioneering in the identification of FGF21 cutoff value greater than 30 pg/dl for the diagnosis of prediabetes with AUC of 1 and 100% for sensitivity, specificity, NPV, PPV, and accuracy. FGF21 reduces blood glucose level and enhances B-cell function and insulin sensitivity through many mechanisms [29–31]. However, the protective effects of FGF21 may be inhibited due to the down regulation of its coreceptor β-Klotho caused by tumor necrosis factor-α in the chronic inflammatory state, leading to FGF21 resistance [32]. As prediabetes and diabetes are inflammatory states [1] they lead to FGF21 resistance with compensatory response similar to IR.

A novel finding in our study was the positive association of FGF21 with studied obesity (BMI, WHR, and waist circumference) and glycemic parameters (FPG, 2-hpp PGHBA1c), IR indices, lipid profile, and hepatic enzymes among prediabetic patients. Diabetic patients and entire groups showed nearly comparable correlations to prediabetic patients. Predictors of FGF21 levels were fasting glucose, fasting insulin, and TG in the prediabetic and diabetic patients groups, in addition to adiposity (BMI and WHR) in prediabetic patients and ACR, age, and SBP in diabetic patients.

To our knowledge, the association of these previously mentioned studied parameters with FGF21 level is still not clear, with conflicting data among diabetic, healthy, and other metabolic disorders. Some of these associations in the current study were previously reported by Chen et al. [5] in a cohort study of Chinese population involving the entire population with normal and IGT and diabetes and in healthy population [33,34]. They reported that serum FGF21 correlated positively with adiposity, BP, glycemic parameters, IR, TC, TG, and LDL-C and inversely with HDL-C level. Moreover, they described that TG and SBP were independent predictors to serum FGF21. However, there are conflicting data in a recent healthy population, except for TG levels [35].

Studies among diabetic patients showed diverse results. Cheng et al. [27] reported that serum FGF21 correlated positively and significantly with age, WHR, SBP, FPG, Hb A1c, and HOMA-IR. Fasting insulin, HOMA-IR, and LDL were independent contributing factors influencing serum FGF21 levels. Eto et al. [36] showed that FGF21 levels were associated with adiposity in univariate analysis, and that the use of fibrates, TG levels, and creatinine levels were significantly correlated and strong contributors to its level among diabetic patients under treatment. In a recent study among newly diagnosed DMT2, adiposity, FPG, and serum TG were correlated positively to FGF21, whereas in another study among

long-duration diabetic patients, adiposity, SBP and diastolic BP, and TG were positively correlated, whereas HDL was negatively correlated to FGF21 level only among female participants [37,38].

Among patients with metabolic syndrome (MetS), one study showed a positive correlation with cardiometabolic risk factors in the entire population (the control and MetS groups) with BMI and FPG as independent predictors to FGF21 [39]. In contrast, another recent study did not find these associations with exception of TG, which was only associated but not predictor [35]. Association of FGF21 with adiposity, IR, or lipid profile among PCOS is still debate [40,41].

Experimental, cellular, and pharmacological studies showed regulatory roles of FGF21 in glucose and lipid metabolism and energy balance and BP. FGF21 exerts antiobesity effects through the regulation of energy expenditure in brown adipose tissue by stimulating sympathetic nerve activity [42]. Several pieces of evidence confirmed the interplay of obesity and FGF21 levels. FGF21 concentrations increased in obesity and decreased with acute weight loss and among those with anorexia nervosa [43–45]. In recent times, FGF21 may be a promising therapeutic target in obesity-related diseases [46].

There was increased FGF21 in primary (HTN [47] and among those with combined either coronary heart disease (CHD)–HTN or obesity–HTN than among those with CHD or obesity alone [43,48]. FGF21 administration had a hypotensive effect in an animal study [49]. Therefore, the association of FGF21 level with BP in our study can be attributed to its key role in the pathophysiological progress and neurocontrol of BP independent on its metabolic effects as its receptors were identified in the hypothalamus [50].

FGF21 regulates fatty acid oxidation and lipogenesis in the liver [51] and adjusts TG/fatty acid cycle in adipose tissue [52]. FGF21 analog improved the lipid profile of obese individuals with type 2 diabetes [53]. Recent studies have shown that TG-induced endoplasmic reticulum stress stimulates FGF21 expression in hepatocytes and serum levels of FGF21 [54]; this may explain the relationship between FGF21 levels and lipid profile, particularly TG, in our study.

Insulin stimulates FGF21 expression in human skeletal muscle. Thus, FGF21 is an insulin-regulated myokine. Artificial hyperinsulinemia in humans is associated with high FGF21 levels. It is increased with DMT2 and other IR states but not in DMT1 [55]. It is associated with hepatic and skeletal muscle IR among patients with impaired glucose metabolism [26]. All these data explain fasting insulin as a predictor of FGF21 level.

Higher serum FGF21 was associated with a significant increase in combined cardiovascular events and mortality among diabetic patients in recent short and long follow-up studies [8,9]. These data support our novel finding – the association of FGF21 level with CVD risk score among diabetic and prediabetic patients assessed using Framingham and ADVANE risk scores, respectively. In recent times, the relevance role of FGF21 in CVD and atherosclerosis has been highlighted in many studies. FGF21 is associated with CHD [56], subclinical atherosclerosis, and carotid atheroma independent of established risk factors among diabetic and nondiabetic patients [37,57]. FGF21 protects against atherosclerosis through direct and indirect effects on vascular pathology. Its deficiency was associated with aggravation of diabetic vascular pathology, including oxidative stress, inflammation, and cell apoptosis in the aorta of diabetic mice [58,59].

NAFLD is considered as a specific manifestation of the metabolic syndrome and affects mainly patients with IGT as we observed in our study [60]. FGF21 deficiency is associated with increased hepatic steatosis and inflammation in early stage in nonalcoholic steatohepatitis (NASH) [61]. In line with previous study among nondiabetic individuals [33], we demonstrate that FGF21 serum levels are positively associated with hepatic enzymes, including AST and ALT. Our results are in accordance with other studies that reported increased levels in NAFLD patients and its clinical use as a noninvasive biomarker to differentiate simple fatty liver and NASH [62]. Increased hepatic expression of FGF21 is a response to FGF21 resistance, and just FGF21 resistance may be involved in the pathogenesis of NAFLD/NASH [63].

FGF21 has recently been identified as a novel biomarker of progression in diabetic nephropathy. It is progressively increased from early to late diabetic nephropathy [64]. In the present study, prediabetic patients had normoalbuminuria and diabetic patients had microalbuminuria. In line with An *et al.* [65], we found that serum FGF21 positively correlated with ACR in diabetic patients. However, an inverse correlation was observed in prediabetes, an aspect not explored before. We hypothesized that, in normoalbuminuric range, FGF21 can delay the progression of renal injury and the development of microalbuminuria owing to its renoprotective effects. It

decreased renal apoptosis and suppressed diabetes-induced renal inflammation, oxidative stress, and fibrosis in animal models [66]. Progression of renal injury and development of microalbuminuria associated with impaired FGF21 signaling and increased FGF21 levels might simply reflect compensatory responses, and reflect on the severity of the underlying renal inflammation and injury in type 2 diabetes [64]. These data support our finding of ACR as a predictor of FGF21 levels in diabetic patients.

## Conclusion

The increase in FGF21 level may be a potential protective factor against lipid and carbohydrate metabolism disorders and IR, and reduces hepatic lipid accumulation in these patients and reflect compensatory mechanism to FGF21 resistance. We demonstrate that FGF21 is an excellent marker for the diagnosis of prediabetes. It is positively associated with CVD risk score. It is positively and independently correlated with facets of the metabolic syndrome, ACR and IR. Finally, FGF21 is a novel linkage factor between metabolic disorders and circulatory system in prediabetic and diabetic population.

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**Conflict of interest**

There are no conflicts of interest.

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