Fibroblast Growth Factor 21 as a Marker of Prediabetes in Patients with Non-alcoholic Fatty Liver Disease

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ABSTRACT

Background: *Fibroblast growth factor 21 is a peptide primarily secreted by the liver in response to peroxisome proliferator-activated receptor-α activation which plays an important role in regulating carbohydrate and lipid metabolism. This study investigated the association between fibroblast growth factor 21 and prediabetes in obese patients with non-alcoholic fatty liver disease in adult population.*

Methods: *A total of 85 obese non-alcoholic fatty liver disease patients without (n = 49) and with prediabetes (n = 36) were included. Serum fibroblast growth factor 21 levels were determined by enzyme-linked immunosorbent assay.*

Results: *Higher fibroblast growth factor 21 serum levels were observed in patients with prediabetes, metabolic syndrome, dyslipidemia, and insulin resistance. There were significant correlations between fibroblast growth factor 21 and waist-to-stature ratio, visceral adiposity index, triglycerides, very low-density lipoproteins, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), Quantitative Insulin Sensitivity Check Index, and Stumvoll index of insulin sensitivity. Fibroblast growth factor 21 level ≥320 pg/mL was associated with a 4.2-fold higher risk of prediabetes and ≥270 pg/mL for metabolic syndrome approximately 4 times.*

Conclusion: *Fibroblast growth factor 21 is associated with increased risk for prediabetes, metabolic syndrome, and insulin resistance in obese patients with non-alcoholic fatty liver disease.*

Keywords: *FGF21, insulin resistance, metabolic syndrome, NAFLD, prediabetes*

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases, affecting around 25% of the worldwide population.[1,2] Non-alcoholic fatty liver disease is a continuum of liver abnormalities, from non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma. Currently, NAFLD has been confirmed to be closely associated with obesity, dyslipidemia, insulin resistance (IR), and prediabetes forming together a metabolic syndrome (MetS), and there is an international collaborative effort on renaming NAFLD as metabolic associated fatty liver disease (MAFLD).[3-5]

Under the status of NAFLD, MetS and pro-inflammatory conditions inevitably increase the abnormal secretion of cytokines. It has been shown that the liver can affect the metabolism of glucose and lipids by releasing hepatokines, and NAFLD is associated with their altered production. Some of them may directly affect the risk of type 2 diabetes mellitus (T2DM) by adverse effects on hepatic gluconeogenesis, glycogen synthesis, and insulin signaling. In addition, hepatokines appear to be considered as biomarkers of ectopic fat accumulation in the liver, as well as markers of disease progression. It is suggested that some of them may be targeted for prevention and treatment of diseases associated with IR, including prediabetes and NAFLD.[6]

Fibroblast growth factor 21 (FGF21), belonging to the FGF superfamily, is a novel discovered peptide that is synthesized predominantly in the liver and plays an important role in the regulation of hepatic glucose production and regulation of fatty acid oxidation, possibly through activation of peroxisome proliferator-activated receptor-α (PPARα) and enhancing mitochondrial function, but some of these data remain controversial. In a large multiethnical study, elevated FGF21 levels have been associated with MetS.[7] There is supporting evidence that

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FGF21 is significantly increased in several diseases, such as obesity, T2DM, and coronary artery diseases, modulating the pathological development of these diseases.[8] A consistent finding in many published data has shown that circulating FGF21 levels are increased in IR, impaired glucose tolerance (IGT), hypertriglyceridemia, and hepatic injury. In liver, FGF21 expression is regulated by PPARα, liver X receptor, and the postprandial status.[9] Current evidence shows that serum FGF21 levels increase in patients with NAFLD. Dushay et al[10] found that both the hepatic FGF21 gene expression and the serum FGF21 levels were elevated in Spanish NAFLD patients, suggesting that FGF21 might be a novel biomarker for NAFLD. Another study reported that serum levels of FGF21 were inversely correlated with the severity of fibrosis in Italian NASH children.[11] A prospective observational study in patients undergoing a low carbohydrate dietary intervention for weight loss showed that baseline circulating FGF21 was a negative predictor of liver fat reduction.[11,12] The authors suggest that individuals with lower serum FGF21 might be more sensitive to its hepatoprotective action achieving a more improvement in liver fat accumulation on therapeutic intervention, and FGF21 could play an active role in NAFLD amelioration, especially in those individuals whose metabolic disturbance is not a so-called FGF21-resistant state.[12] Nevertheless, taken together, these results reveal that serum levels of FGF21 are associated with the pathogenesis of NAFLD and MetS-related disorders.

The objective of this study was to investigate the relationship between FGF21 and prediabetes as well as with other components of MetS in obese patients with NAFLD.

MATERIALS AND METHODS

Study Population

A total of 85 Bulgarian persons with NAFLD aged 27-78 years (mean age 50.2 ± 10.9 years) recruited in a Clinic of Endocrinology, University Hospital "Alexandrovska," Sofia setting, participated in the study. Inclusion criteria were ultrasound-based diagnosis of NAFLD with enhanced liver fibrosis (ELF) score < 9.8[13]; fibrosis stage 4 score (FIB-4) < 1.30; NAFLD fibrosis score (NFS) < 0.675; obesity (body mass index (BMI) ≥ 30 kg/m^2); normal glucose metabolism, or prediabetes. Prediabetes is defined specifically as IGT and/or impaired fasting glucose. According to the American Diabetes Association, IGT is defined as a 2-hour plasma glucose value in the 75 g oral glucose tolerance test (OGTT) of 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L). Impaired fasting glucose is defined as a fasting plasma glucose of 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L). Prediabetes can also be defined as a hemoglobin A1c (HbA1c) of 5.7-6.4% (39–46 mmol/mol), but we used only the result of OGTT.[14] Study participants were not included if any of the following criteria were present: secondary cause of hepatic steatosis and significant alcohol consumption >20 g daily for women and >30 g daily for men, T2DM, proven neoplasia, chronic kidney disease (estimated glomerular filtration rate (eGFR) calculated by chronic kidney disease epidemiology collaboration (CKD-EPI) formula <60 mL/min/1.73 m^2), heart failure, hypothyroidism. All subjects were divided into 2 groups. Group 1 included 49 obese NAFLD patients without glucose metabolism disturbance. Group 2 included 36 obese NAFLD patients with prediabetes. The project was approved by the University ethics committee for clinical studies, and all included patients signed informed consent for participation in the study.

The following study methods were used: Anthropometric parameters such as weight (kg), height (m), BMI (kg/m^2), waist and hip circumference (cm), waist-to-hip ratio (WHR), and waist-to-stature ratio (WSR) were calculated. Visceral adiposity index (VAI) was calculated using the following formula: VAI = (waist circumference (WC)/(36.85 + (1.89 × BMI))) × (triglycerides (TG)/0.81) × (1.52/high-density lipoprotein (HDL) for females and VAI = (WC/(39.68 + (1.88 × BMI))) × (TG/1.03) × (1.31/HDL) for males.[15] Percentage body fat (%) was measured by means of body impedance analysis by a TANITA TBF-215 GS Body Composition Analyzer in fasting state. MetS was diagnosed following the International Diabetes Federation (IDF) criteria.[16] A standard OGTT with measurement of glucose (mmol/L) and insulin (μU/mL) on 0 minute (glucose 0 and insulin 0), 60 minutes (glucose 60 and insulin 60), and 120 minutes (glucose 120, insulin 120), as well as other laboratory tests were performed in the Central Laboratory of the Alexandrovska University Hospital, which is referent center for Bulgaria. Homeostatic model assessment for IR (HOMA-IR) was calculated using the following formula: HOMA-IR = fasting plasma glucose (mmol/L) × fasting serum insulin (mIU/L)/22.5. Insulin resistance was defined if HOMA-IR > 2.5 The Quantitative Insulin Sensitivity Check Index [QUICKI Index × 1/log (fasting plasma glucose in mmol/L) × log (fasting insulin in mIU/mL)], as well as Stumvoll Index of insulin sensitivity [ISI Stumvoll, based on plasma glucose (mmol/L) and insulin (IU/L) concentrations during OGTT with or without demographic parameters (BMI, age)] were also calculated.[17]

The ELF score was measured using an ADIVA Centaur automated system. The ELF score was calculated using the published algorithm combining tissue inhibitor of

metallo-proteinases-1, amino-terminal propeptide of type III procollagen, and hyaluronic acid values. Enhanced liver fibrosis, NFS, and FIB-4 were calculated to exclude advanced fibrosis. Abdominal ultrasonography was performed by an experienced gastroenterologist who was blinded to clinical presentation and laboratory results. Hepatic steatosis was defined as a diffuse increase of fine echoes in the liver parenchyma compared with that in the kidney or spleen parenchyma based on standard criteria.

Measurement of serum FGF21 levels was performed by enzyme-linked immunosorbent assay (Human Fibroblast Growth Factor, ELISA kit, Biovendor, Czech Republic).

Statistical Analysis

All analyses were performed with Statistical Package for Social Sciences version 25.0 (SPSS, Chicago, Ill, USA). Normally distributed data were expressed as means ± standard deviation (SD). Data that were not normally distributed, as determined using Kolmogorov–Smirnov test, were logarithmically transformed before analysis and expressed as median with interquartile range. Student's unpaired *t*-test was used for comparison between 2 groups. One-factor dispersion analysis of variance for several independent samples, correlation analysis for linear dependence between quantitative signs, binary logistics regression for evaluation of the impact of the researched factors, and receiver operating characteristic (ROC) curve for threshold levels of quantitative signs for classification of certain conditions were used for validation of screening tests. The level of significance for rejecting the null hypothesis was $P < .05$.

RESULTS

Both groups were similar in age, BMI, waist circumference, hip circumference, WHR, WSR, and percentage of fat mass. Of the patients with NAFLD without carbohydrate disturbances, 89% were women, 60.9% had hypertension, 43.5% had dyslipidemia, and 52.2% were with MetS. In the group with NAFLD and prediabetes, female prevalence exceeded again than that of males with 80%. Patients with hypertension were 91.4%, those with dyslipidemia were 71.4%, and almost 95% from the study group were with MetS. A significant higher levels of diastolic blood pressure, TG, very low-density lipoprotein (VLDL), VAI, blood glucose and insulin from OGTT, HOMA-IR, as well as lower levels of HDL were found in obese NAFLD patients with prediabetes rather than in the group without prediabetes (Tables 1 and 2). Statistical analyses were adjusted for age. The obese NAFLD patients with prediabetes had significantly higher levels of FGF21

Table 1. Comparative Analysis Between the Groups According to Age and Anthropometric Parameters

	Without Prediabetes (n = 49)		With Prediabetes (n = 36)		
Parameters	$\bar{X}$	SD	$\bar{X}$	SD	*P*
Age (years)	49.9	10.6	54.2	8.4	.048
BMI (kg/m^2)	36.9	6.1	37.7	5.5	.405
Waist (cm)	110.9	14.5	114.5	11.6	.119
Hip (cm)	118.2	12.5	149.9	170.62	.354
WHR	0.94	0.17	0.93	0.17	.550
WSR	0.67	0.08	0.69	0.06	.194
VAI	2.71	1.98	10.74	41.21	.001
Fat tissue (%)	45.31	5.55	46.06	6.65	.325

BMI, body mass index; WHR, waist-to-hip ratio; WSR, waist-to-stature ratio; VAI, visceral adiposity tissue; SD, standard deviation.

Table 2. Comparative Analysis Between the Groups According to Arterial Pressure and Metabolic Parameters

	Without Prediabetes (n = 49)		With Prediabetes (n = 36)		
Parameters	$\bar{X}$	SD	$\bar{X}$	SD	*P*
SBP (mmHg)	130.82	16.15	137.36	16.10	.149
DBP (mmHg)	79.80	16.01	86.25	10.45	.021
TC (mmol/L)	5.30	0.96	5.29	1.07	.329
HDL (mmol/L)	1.26	0.33	1.12	0.26	.007
LDL (mmol/L)	3.25	0.85	3.19	0.99	.699
VLDL (mmol/L)	0.67	0.28	0.94	0.47	<.001
TG (mmol/L)	1.64	0.89	2.27	1.13	<.001
Glu 0 (mmol/L)	5.33	0.51	6.33	1.19	<.001
Glu 60 (mmol/L)	7.70	1.76	10.51	2.21	<.001
Glu 120 (mmol/L)	5.80	1.23	8.17	1.77	<.001
Insulin 0 (μU/mL)	14.13	7.77	29.67	39.91	<.001
Insulin 60 (μU/mL)	91.70	58.28	146.46	139.51	.024
Insulin 120 (μU/mL)	53.89	47.64	146.92	177.52	<.001
HOMA-IR	3.36	1.95	8.13	11.45	<.001
QUICKI Index	2.33	1.55	1.28	0.40	<.001
ISI Stumwoll	0.05	0.04	0.01	0.08	<.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low-density lipoproteins; TG, triglycerides; Glu0, glucose at the 0 minute; Glu 60, glucose at 60 minutes; Glu120, glucose at 120 minutes; Insulin0, insulin at the 0 minute; Insulin 60, insulin at 60 minutes; Insulin 120, insulin at 120 minutes; HOMA-IR, homeostatic model assessment of insulin resistance; SD, standard deviation; QUICKI Index, Quantitative Insulin Sensitivity Check Index; ISI Stumvoll, Stumvoll Index of insulin sensitivity; TC, total cholesterol.

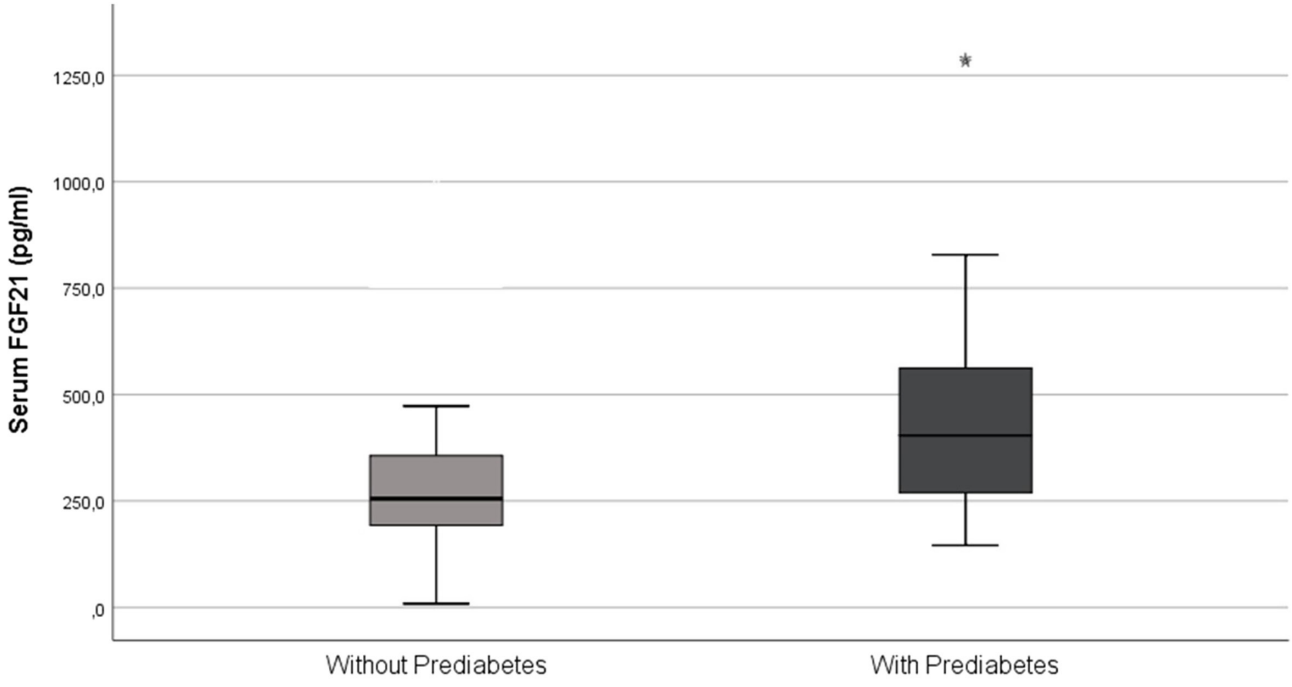

Figure 1. Serum FGF-21 between NAFLD patients with and without prediabetes (450.98 ± 294.82 vs. 360.6 ± 298.09 pg/mL, *P* = .010). FGF-21, fibroblast growth factor 21; NAFLD, non-alcoholic fatty liver disease.

compared to those patients without prediabetes (450.98 ± 294.82 vs. 360.6 ± 298.09 pg/mL, *P* = .010) (Figure 1). Higher mean levels of FGF21 were also observed in patients with MetS, compared to patients without MetS (n = 58; 457.78 ± 312.24 vs. n = 24; 261.33 ± 132.82 pg/mL, *P* < .001). The patients with dyslipidemia and IR had significantly higher levels of FGF21 (n = 45; 470.9 ± 312.82 vs. n = 37; 314 ± 224.54, *P* = .004) and (n = 60; 439.75 ± 302.37 vs. n = 22; 303.46 ± 215.78, *P* = .020, respectively). The comparative analysis of patients with and without arterial hypertension did not show any statistically significant difference in the levels of FGF21. We observed moderate positive correlation between FGF21 and TG (0.352, *P* < .01), VLDL (0.384, *P* < .01), ALT (0.250, *P* < .05), GGT (0.378, *P* < .01), glucose 0 minute (0.258, *P* < .005), glucose 60 minute (0.342, *P* < 0.01), and glucose 120 minute (0.379, *P* < .001). There was a moderate negative correlation between FGF21 and indicators of insulin sensitivity QUICKI Index and Stumvoll Index (–0.263, *P* < .05 and –0.253, *P* < .05, respectively).

A multiple binary logistic regression analysis was conducted to assess whether the FGF21 has any predictive value for the risk of prediabetes. The ROC curves method was applied to find a threshold value (Figure 2). FGF21 ≥ 320 pg/mL differentiate patients with

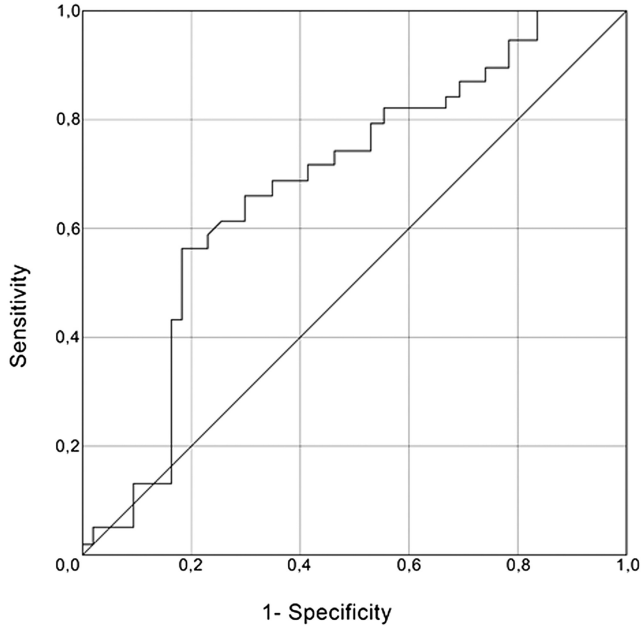

Figure 2. ROC curve of FGF21 (area under the curve 0.679, *P* = .005) to determine the threshold value for differentiation between patients with and without prediabetes. FGF21, fibroblast growth factor 21; ROC, receiver operating characteristic curve.

and without prediabetes with high percentages. Patients with FGF21 ≥ 320 pg/mL compared to those with lower values had about 4.2-fold higher risk of prediabetes (95% CI 1.665-10.592; *P* = .002) (Table 3). We also assessed the predictive value of FGF21 for the occurrence of MetS and IR. Threshold value of FGF21 ≥ 270 pg/mL (ROC analysis: AUC 0.713, *P* = .003) differentiated patients with and without MetS with acceptable sensitivity 71%, specificity 63%, positive predictive value 82%, negative predictive value 74%, and precision 68%. The FGF21 ≥ 270 pg/mL increased the risk for MetS approximately 4 times (odds ratio (OR) 4.020, 95% CI 1.477-10.941, *P* = .006).

About the same threshold value of FGF21 ≥ 260 pg/mL (ROC analysis: AUC 0.669, *P* = .20) differentiated patients with and without IR with acceptable percentages: sensitivity 73%, specificity 55%, positive predictive value 81%, negative predictive value 43%, and precision 68%. The FGF21 ≥ 260 pg/mL increased the risk for IR about 3.2 times (OR 3.225, 95% CI 1.167-8.914, *P* = .024).

DISCUSSION

In this study, we provide the first clinical evidence showing that serum levels of FGF21 were elevated in obese patients with NAFLD and prediabetes in Bulgarian population. We excluded some diseases and conditions that may affect our results, such as advanced liver fibrosis, T2DM, impaired renal function, heart failure, and hypothyroidism.

Mean FGF21 serum levels were higher in patients with prediabetes, MetS (independently of carbohydrate disturbances) as well as with dyslipidemia and IR compared to the patients without these metabolic abnormalities. As expected, in the patients with prediabetes, there were more significant abnormalities in the surrogate markers for IR (HOMA-IR) and insulin sensitivity (QUICKI and Stumvoll Index), lipids, and VAI compared to the group of patients without prediabetes.

The metabolic role of FGF21 in animal models is supported by increasing evidence, but the role of FGF21 in human physiology is still controversial. The function of FGF21 was first discovered in the central nervous system as FGF21 could act on nerve cells to regulate circadian rhythm and stimulate the hypothalamic–pituitary–adrenal axis and increase corticosterone secretion which subsequently promoted the process of gluconeogenesis in livers.[18] There is evidence from animal-based studies suggesting FGF21 as a potent metabolic regulator with multiple beneficial effects on obesity and diabetes, hepatic lipogenesis, and fatty acid oxidation in hepatocytes.[19,20] Some studies indicate that in rodents, FGF21 increases the expression of the glucose transporter glucose transporter 1 (GLUT-1), thereby increasing glucose uptake in an insulin-independent manner.[19] In obese mice, administration of FGF21 reduces body weight and improves glycemia and hypertriglyceridemia. In diabetic rhesus monkeys, treatment with recombinant FGF21 led to a decline in fasting plasma glucose, insulin, and glucagon and improvement in lipoprotein profiles.[20] Prolonged intake of FGF21 resulted in a significant decrease in hepatic steatosis in diet-induced obese mice.[21] These animal-based studies demonstrated that FGF21 might represent a promising therapeutic agent for diabetes and NAFLD.

Table 3. Odds Ratio and 95% Confidence Intervals of the Studied Predictors for Development of Prediabetes

			95% CI		
Parameter	Comparison	OR	Lower Limit	Upper Limit	*P* value
FGF21	≥320/<320	4.200	1.665	10.592	.002

In a previous study, in patients with biopsy-proven NAFLD, FGF21 levels were significantly increased regardless of the confounding factors. Moreover, steatosis was associated with increased FGF21 levels rather than other histological parameters.[22]

There are a number of findings for the association between FGF21 and T2DM, MetS, and IR. A large meta-analysis by Wang et al[23] shows elevated levels of FGF-21 in patients with overt T2DM compared to controls, with levels correlated with BMI, total cholesterol, and TG. In our study, we found correlations between serum levels of FGF21 and TG, VLDL, ALT and GGT, glucose levels during OGTT, WSR, and surrogate marker for visceral fat (VAI). Confirmation of the sensitivity of FGF21 from our results was that its values increase in direct proportion to the severity of carbohydrate disorders. Moreover, with respect to the predictive value of FGF21 for prediabetes in obese NAFLD patients in those with FGF21 ≥ 320 pg/mL vs. those with lower values, the risk of prediabetes is about 4.2 times higher. Predictive FGF21 values were also found for the risk of MetS and IR, with levels ≥270 pg/mL associated with a 4-fold higher risk for MetS and ≥260 pg/mL with a 3.2-fold higher risk for IR.

Some of our results coincide with the results of other studies.[24] Samms et al[25] reported a high rise in circulating

levels of FGF21 during OGTT and clamping technique that postprandial elevation of FGF21 was actually explained by insulin rather than glucose. On the other hand, there are data on the inverse correlation between FGF21 and the progression of NAFLD.[10,11] Alisi et al[11] found lower levels of FGF21 and FGF19 in children with NAFLD in comparison to a control group, and they were inversely associated with the probability of NASH and fibrosis. They used liver biopsy to diagnose and stage NAFLD which is the gold standard, and this allowed to better realize the NALFD–FGFs relationship by separating simple steatosis from NASH and by separately evaluating fibrosis. Conversely, Barb et al[26] reported that the plasma FGF21 levels were positively correlated with the severity of NASH in patients with obesity and T2DM, and treatment with FGF21 could reduce the prevalence rate of NAFLD.

Furthermore, some authors suggest that elevated levels of FGF21 are likely to have a protective effect against lipid and carbohydrate disorders. Intravenous and subcutaneous administration of FGF21 in experimental models and in people with NAFLD, obesity, and T2DM leads to improvement in hepatic steatosis, reduction in IR and fasting blood glucose.[21,27] These effects are associated with both an improvement of insulin sensitivity and an increase in adiponectin levels. Sanyal et al[28] demonstrated that treatment with subcutaneously administered Pegbelfermin (PEGylated human fibroblast growth factor 21) for 16 weeks was well tolerated and significantly reduced hepatic fat fraction in patients with non-alcoholic steatohepatitis. This does not imply that increased levels of FGF21 present in patients with NASH are actually protective, as this can only be hypothesized. Clearly the absence of FGF21 in both less and more physiological rodent models of NAFLD is associated with a worsening of the liver pathology, but the topic is still open for discussion.[29] Moreover, FGF21 physiology seems to be very different in rodents as compared to humans in terms of glucose and lipid homeostasis. In fact, Fisher et al[30] proposed that there might be a state of FGF21 resistance similar to insulin or leptin resistance in obesity, to justify the higher levels found in metabolically unhealthy patients. Future studies are needed to use FGF21 in the treatment of NAFLD and related metabolic disorders, including T2DM and obesity.

There are several limitations in our study, including the relatively small sample size and cross-sectional design, which limit the ability to evaluate the dynamics of FGF21 and other risk factors of NAFLD and MetS over a period of time. We used ultrasound to diagnose NAFLD and other non-invasive methods instead of a liver biopsy, which is the gold standard. Some strengths of this study include the homogeneity of the study population, the early stage of NAFLD, the presence of a control group, and the lack of potential interfering medications.

CONCLUSIONS

Fibroblast growth factor 21 is increased in obese NAFLD patients with prediabetes and/or MetS or dyslipidemia as compared to controls. It correlates with surrogate markers for visceral adiposity, IR, as well as glucose, TG, and hepatic enzymes. Fibroblast growth factor 21 is associated with an increased risk for prediabetes, MetS, and IR.

Ethics Committee Approval: The study was approved by the medical ethics committee of Medical University - Sofia.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – V.K., Z.K., Y.A.; Design – V.K., A.G.; Supervision – Z.K., L.M., Resources – V.K., I.N., N.C., I.I., Materials – Y.A.; Data Collection and/or Processing – I.N., V.K., A.G., I.I.; Analysis and/or Interpretation – Y.A., V.K., Z.K.; Literature Search – V.K., A.G., I.N.; Writing Manuscript – V.K.; Critical Review – Y.A., Z.K., L.M.

Declaration of Interests: The authors have no conflict of interest to declare.

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