Diabetes & Metabolic Syndrome: Clinical Research & Reviews 13 (2019) 882–888

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

Fibroblast growth factor 21 association with subclinical atherosclerosis and arterial stiffness in type 2 diabetes

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ARTICLE INFO

Article history:
Received 16 November 2018
Accepted 18 December 2018

ABSTRACT

Aim: To evaluate the association of serum FGF21 with subclinical atherosclerosis and pulse wave velocity, a marker of arterial stiffness, in type 2 diabetes Egyptian patients.
Patients and methods: Fasting serum FGF21 was measured in 120 type 2 diabetes patients without clinical atherosclerotic cardiovascular disease (mean age 51.1 ± 7.7 years; 63.3 women). In addition to basic laboratory tests, serum adiponectin and ultrasonographic examination of CIMT, ankle brachial index (ABI) and carotid-femoral pulse wave velocity (cfPWV) were performed.
Results: Patients with subclinical atherosclerosis have higher serum FGF21 than those without (218 ± 66.8 pg/mL Vs 170 ± 43.1 pg/mL, $P < 0.001$). FGF21 correlated positively with CIMT and cfPWV ($P < 0.001$) regardless of patient gender. In logistic regression analysis, circulating FGF21 was found to be an independent predictor for subclinical atherosclerosis ($P = 0.023$) in addition to dyslipidemia, hypertension and adiponectin. FGF21 was also found to be an independent determinant of cfPWV in stepwise multiple regression analysis. ROC curve analysis was done and cutoff high risk FGF21 level of 184 pg/mL for the prediction of subclinical atherosclerosis with a sensitivity and specificity of 66.7%.
Conclusions: Serum FGF21 levels correlated with carotid intima media thickness and predict subclinical atherosclerosis. Serum FGF21 is also correlated positively with cfPWV and arterial stiffness in type 2 diabetes patients.

1. Introduction

The prevalence of diabetes mellitus is increasing significantly worldwide. Patients with diabetes are at higher risk of microvascular and macrovascular complications. Cardiovascular diseases (CVD) are the most prevalent cause of mortality and morbidity in diabetes. The International Diabetes Federation (IDF) [1] listed Egypt among top 10 countries in diabetes prevalence. Prevalence of diabetes in Egypt is around 15.56% in adults between 20 and 79 years of age [2].

Atherosclerosis is a chronic, progressive, multifactorial inflammatory disease of the medium to large-sized arteries with long asymptomatic stage. It develops over years as a subclinical condition and at last it becomes expressed clinically as cardiovascular disease, cerebrovascular disease, or peripheral arterial disease [3]. Subclinical atherosclerosis, refers to the early stage of the atherosclerosis process when the cardiovascular disease is not clinically evident. Detection and assessment of cardiovascular risk at the subclinical stage, may help to prevent disease development. This could be done by non-invasive techniques as coronary artery calcium scoring, carotid intima-media thickness (CIMT), carotid plaque burden, pulse wave velocity (PWV) and ankle-brachial index (ABI). All these techniques were found to be predictive of future cardiovascular complications. In addition, a large number of emerging risk markers are continuously developed and proposed as representative measures of atherosclerosis burden [4].

The CIMT reflects the diffuse thickening of the intimal layer seen in atherosclerosis and has been validated as a surrogate measure of

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https://doi.org/10.1016/j.dsx.2018.12.007
1871-4021/

subclinical atherosclerotic disease [5]. Pulse wave velocity is another valuable noninvasive marker of atherosclerosis. It mainly reflects arterial stiffness and it is a determinant of severity of arterial damages and a prognostic measure for development of atherosclerotic events in diabetes and hypertension [6]. Arterial stiffness is an age-related progressive increase in rigidity of the artery wall, a process that is a shared consequence of numerous diseases including diabetes mellitus, hypertension, metabolic syndrome and chronic kidney disease, among others [7]. Different studies reported an increase in arterial stiffness in diabetes. This increase in arterial stiffness was postulated to be a mechanism linking diabetes to the associated increase in cardiovascular risk [7].

Fibroblast growth factor 21 (FGF21) is a liver-derived circulating hormone mainly produced in the liver and other tissues, such as adipose tissue, pancreas and skeletal muscles [8]. It belongs to the FGF superfamily and bound as a complex with b-klotho protein and FGF receptors (FGFRs) to perform its endocrinal functions. FGF21 is an emerging metabolic regulator of glucose and lipid metabolism with pleotropic roles as a hepatokine, adipokine, and myokine in metabolism and injury protection [9]. Recently, FGF21 was linked to atherosclerosis but the mechanism of this link is still under investigation. Whether the increased serum FGF21 level is the basis for CVD pathogenesis or is induced to protect the heart form CVDs is still under discussion. However, growing evidence indicated that administration of exogenous FGF21 induces preventive effects on most of the CVDs, suggesting that FGF21 is not only a simple marker of cardiovascular risk but also induces a protective effect on the cardiovascular system contributing to a reduction in risk [10].

There is a considerable research on the association of serum FGF21 levels with different cardio-metabolic risk factors, adiposity index, and atherosclerosis in varied populations [11,12]. In T2DM patients, a few studies were reported regarding the association between elevated serum FGF21 levels and carotid atherosclerosis [13,14]. However, only very limited clinical studies regarding associations between serum FGF21 levels and Ankle brachial index (ABI) or pulse wave velocity (PWV) in diabetes patients. To the best of our knowledge this is the first study in which we try to find FGF21 relationship with subclinical atherosclerosis and arterial stiffness and other clinical parameters in Egyptian diabetes patients.

2. Patients and methods

2.1. Study population

This was a cross-sectional study conducted in Alexandria university hospital between February and November 2017. We enrolled 120 patients with T2DM of them 44 men (36.67%) and 76 women. Patients with overt cardiovascular disease or history of revascularization or clinical evidence of peripheral arterial diseases were excluded. Endocrinal or metabolic diseases, type 1 diabetes, gestational diabetes, patients with renal or hepatic impairment were also excluded. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Alexandria ethics committee. A complete history and physical examination were obtained. Body mass index (BMI) defined as weight over height (kg/m^2). Waist circumference (WC), in centimeters, was measured at midway between iliac crest and lower rib margin. Systolic and diastolic blood pressure of the study population were measured using a mercury sphygmomanometer after 10 min of rest.

2.2. Blood sampling

Venous blood samples were collected from all patients after overnight fasting. The levels of total cholesterol, low density lipoprotein-cholesterol (LDL), high density lipoprotein-cholesterol (HDL), triglycerides (TG), fasting plasma glucose (FPG), HbA1c and serum creatinine were measured by suitable laboratory techniques including HPLC for HbA1c. Serum samples stored at −80 °C were used for the determination of FGF21 levels using ELISA assays kit supplied by Biomatik (Ontario, Canada), detection range 7.8–500 pg/mL. Serum total adiponectin was determined by ELISA kits supplied by Thermo Fisher Scientific (Waltham, USA).

2.3. Carotid intima-media thickness

Carotid artery ultrasonography was performed by an experienced radiologist on both sides of the neck using a standardized Doppler ultrasonic device (Arietta S70, Hitachi, USA) with 7.5 Mhz probe. Patients were examined in the supine position and measurement of CIMT was made at a point on the far wall of the common carotid artery, 2 cm proximal to the bifurcation, from a longitudinal scan plane that showed the intima-media boundaries most clearly [15]. Measurements on both sides were performed for each patient and the mean value of 3 measures were used for statistical analysis.

2.4. ABI measurement

Ankle pressure was measured using a standardized doppler ultrasonic device (5Mhz; Nicolet Elite 200R, VIASYS Healthcare Inc, USA). ABI was calculated as the ratio of the systolic pressure from posterior tibial artery at the ankle to the systolic pressure from the brachial artery. Pressure in each leg was measured and ABI was calculated separately for each leg. Peripheral arterial disease (PAD) was defined if ABI was <0.9 in at least one leg. The lower ABI between the two legs was used to define PAD [16].

2.5. Carotid to femoral arterial pulse wave velocity (cfPWV) measurement

We used pulsed Doppler ultrasound synchronized with ECG using Arietta S70, Hitachi, USA. The examination was performed as it was described previously [17]. Doppler PWV was measured in supine position after resting for at least 10 min. Flow waveforms were obtained at the right common carotid artery and right femoral artery by the Doppler ultrasound and ECG was also recorded. The time differences between the R wave of the ECG signal and the onset of the flow wave at the two sites were calculated and averaged over three consecutive cycles. The distance between both carotid and femoral points was measured by tape and multiplied by 0.8 [18]. Doppler PWV was defined as cfPWV = 0.8×carotid to femoral distance/Time (meter per seconds). Normal PWV was defined if cfPWV ≤ 10 m/s according to the European Society of Hypertension and the European Society of Cardiology (ESH-ESC) 2013 Guidelines [19].

Subclinical atherosclerosis in this study was considered if patient has CIMT > 9.0 mm and/or ABI <0.9 in the absence of clinical criteria of atherosclerotic cardiovascular diseases.

2.6. Statistical analysis

Statistical analyses were performed by using SPSS 23 system for Windows (SPSS Inc. Chicago, IL, USA). Continuous variables were expressed as means ± standard deviation. Categorical variables were expressed as percentages. Continuous variables were examined for normality of distribution by graphical representation, skewness and kurtosis measures. Transformation were performed when required for analysis. Student's *t*-test or the Mann-Whitney *U*

test were used as appropriate in comparing group means of continuous variables. Correlation analysis was used for evaluating the correlations between FGF21 and other variables. To determine the predictors of subclinical atherosclerosis, variables were selected for logistic regression analysis. Stepwise multiple regression analysis was performed to find predictors of arterial stiffness. Receiver operating characteristic (ROC) analysis was quantified by computing the area under the curve and 95% confidence interval. Two-tailed $P < 0.05$ indicated statistical significance.

3. Results

The baseline demographics, anthropometric and biochemical findings of study participants are summarized in Table 1. In total, this study consisted of 120 participants (mean age: 51.1 ± 7.7 years). There were 44 (36.7%) men and 76 (63.3%) women in the total group. Men and women were equally distributed and have similar FGF21 level ($P = 0.063$). In comparison to men, women, had comparable percentage of dyslipidemia, hypertension and subclinical atherosclerosis. For the cardiovascular risk factors, men are more smoker ($P < 0.001$). Women have longer duration of diabetes, higher serum cholesterol (P= <0.001), higher LDL ($P = 0.001$), higher ALT ($P = 0.003$) and lower GFR ($P < 0.001$).

In order to find out the influencing factors for FGF21, bivariate correlation analysis of clinical and biochemical parameters with FGF21 were undertaken Table 2, Fig. 1A–C. FGF21 in men was correlated positively with age ($P = 0.016$), WC ($P = 0.010$), duration of diabetes ($P = 0.003$), FPG ($P = 0.014$), cfPWV ($P = 0.003$), and carotid IMT ($P = 0.002$). In addition, FGF21 was negatively correlated with eGFR ($P = 0.014$), HDL ($P = 0.003$) and Adiponectin ($P = 0.004$). In women, FGF21 was correlated positively with FPG ($P = 0.005$), HbA1c ($P = 0.001$), creatinine ($P = 0.033$), total cholesterol ($P = 0.023$), TGD ($P = 0.021$), systolic BP ($P = 0.028$), cfPWV ($P = 0.005$), and carotid IMT ($P < 0.001$). Furthermore, FGF21 was negatively correlated with HDL ($P = 0.011$), Adiponectin ($P = 0.003$) and ABI ($P = 0.002$) in women.

When we compared serum FGF21 levels between patients with subclinical atherosclerosis and those without, we noticeably found patients with subclinical atherosclerosis have higher FGF21 levels (218 ± 66.8 pg/mL Vs 170 ± 43.1 pg/mL, $P < 0.001$). To define subclinical atherosclerosis, we divide the T2DM patients into two groups depending on their CIMT and ABI. In logistic regression analysis Table 3. The predictors of subclinical atherosclerosis in type 2 diabetes mellitus were dyslipidemia, hypertension, FGF21 and adiponectin. Increased circulating FGF21 was found to be an

Table 2
Correlation between FGF21 and different variables.

	Overall (n = 120)		Men (n = 44)		Women (n = 76)	
	r	*P* value	r	*P* value	r	*P* value
Age	.223	**.014**	.363	**.016**	.137	.239
BMI	.225	**.013**	.271	.075	.176	.127
WC	.245	**.007**	.386	**.010**	.203	.079
Duration	.299	**.001**	.436	**.003**	.159	.169
FPG	.340	**.000**	.367	**.014**	.316	**.005**
HbA1c	.262	**.004**	.076	.622	.379	**.001**
GFR	-.299	**.001**	-.369	**.014**	-.203	.078
Insulin	.158	.084	.182	.238	.139	.233
LDL	.194	**.033**	.166	.282	.140	.227
HDL	-.328	**.000**	-.433	**.003**	-.289	**.011**
Triglyceride	.252	**.006**	.252	.099	.265	**.021**
SBP	.204	**.025**	.122	.431	.252	**.028**
DBP	.209	**.022**	.220	.151	.204	.078
Adiponectin	-.399	**<.001**	-.421	**.004**	-.338	**.003**
CIMT	.452	**<.001**	.450	**.002**	.439	**.000**
ABI	-.315	**<.001**	-.171	.268	-.355	**.002**
cfPWV	.368	**<.001**	.432	**.003**	.316	**.005**

r = correlation coefficient, other abbreviations as in Table 1.
Bold values means a significant P value.

Table 1
Baseline characteristics of study participants.

	Overall (n = 120)	Men (n = 44)	Women (n = 76)	P value
	Mean ± SD	Mean ± SD	Mean ± SD	
Smokers (%)	16 (13.3%)	14 (31.8%)	2 (2.6%)	**<.001**
Hypertensive (%)	64(53.3%)	29 (65.9%)	40 (52.6%)	.156
Dyslipidemia (%)	69(57.5%)	20 (45.5%)	45 (59.2%)	.145
Family history (%)	93 (77.5%)	36 (81.8%)	57 (75%)	.389
SCA (%)	42 (35%)	16 (36.4%)	26 (34.2%)	.812
Age (years)	51.1 ± 7.7	50.3 ± 7.4	51.6 ± 7.9	.239
BMI (kg/m^2)	30.5 ± 4.2	29.5 ± 4.7	31 ± 3.8	.092
WC (cm)	106.9 ± 8.7	104.9 ± 11.9	108 ± 6	.668
Duration (years)	5.3 ± 3.9	3.6 ± 3.5	6.3 ± 3.8	**<.001**
FPG (mg/dL)	206.6 ± 82.6	205.5 ± 93.6	207.2 ± 76	.549
HbA1c (%)	9.4 ± 1.7	9.1 ± 1.9	9.6 ± 1.5	.085
ALT (IU/L)	29.9 ± 10.14	26.9 ± 8.4	31.7 ± 10.7	**.003**
Creatinine (mg/dL)	.76 ± .13	.8 ± .14	.74 ± .12	**.008**
eGFR	102.4 ± 20.2	113.5 ± 23.5	95.9 ± 14.7	**.000**
Insulin (μU/ml)	18.9 ± 10.7	17.3 ± 10.7	19.9 ± 10.7	.144
Total Cholesterol (mg/dL)	202.2 ± 37.5	187.3 ± 38.5	210.8 ± 34.2	**.000**
LDL (mg/dL)	130.2 ± 40.5	118.3 ± 47.6	137.1 ± 34.3	**.001**
HDL (mg/dL)	41.5 ± 7.6	41.9 ± 7.1	41.3 ± 7.9	.602
Triglyceride (mg/dL)	187.6 ± 68.3	185.4 ± 72.8	188.8 ± 66	.931
SBP (mm Hg)	139.1 ± 12.2	141.1 ± 11.5	137.6 ± 12.4	.064
DBP (mm Hg)	83.6 ± 7.1	84.9 ± 8.1	82.9 ± 6.4	.217
FGF21 (pg/mL)	185.6 ± 55.6	174 ± 54.7	192.3 ± 55.4	.063
Adiponectin (ng/mL)	7.04 ± 2.6	7.2 ± 2.5	6.9 ± 2.6	.618
CIMT (mm)	.85 ± .17	.82 ± .17	.86 ± .17	.264
ABI	1.03 ± .15	1.03 ± .17	1.03 ± .15	.913
cfPWV (m/s)	13.1 ± 3.2	13.1 ± 3.9	13.1 ± 2.8	.533

Values are expressed as number (%), mean (±SD); ABI, ankle brachial index; ALT, alanine transaminase; cfPWV, carotid femoral pulse wave velocity; CIMT, carotid intima media thickness; DBP, diastolic blood pressure; FGF21, fibroblast growth factor 21; FPG, fasting plasma glucose; SBP, systolic blood pressure; SCA, subclinical atherosclerosis; WC, waist circumference.

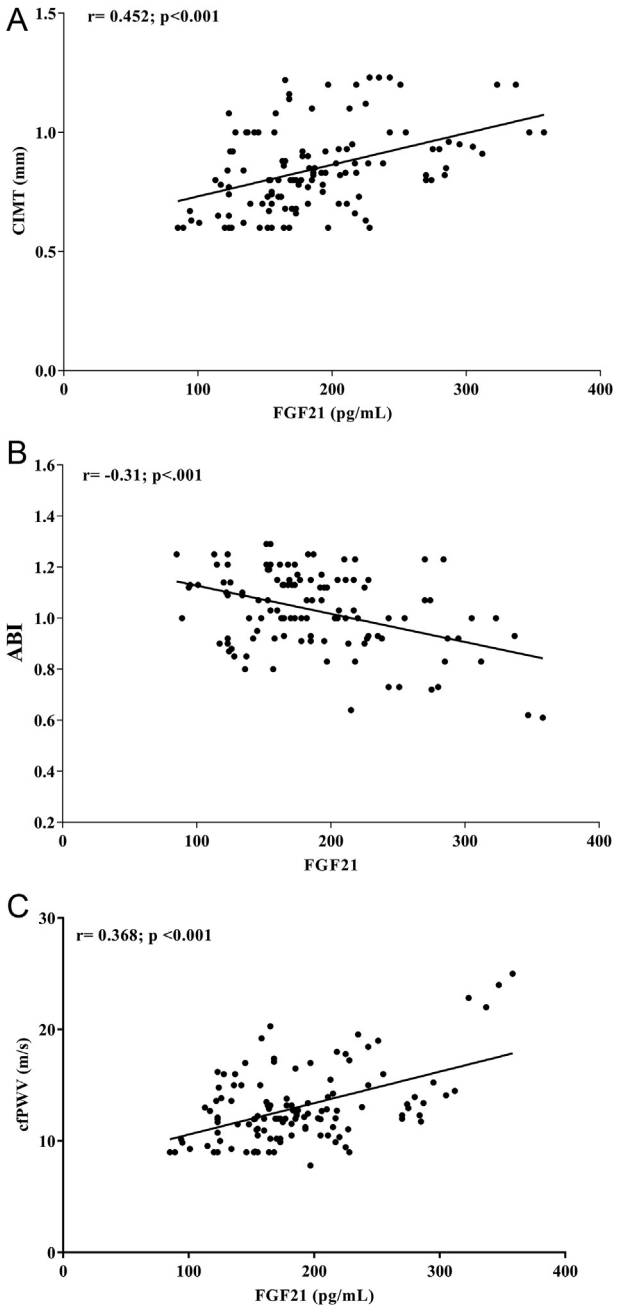

Fig. 1. Correlation of FGF21 with CIMT (A.), Ankle brachial index (B.) and carotid femoral pulse wave velocity (C.).

independent predictor for subclinical atherosclerosis logistic regression model, (P = 0.023). In stepwise multiple regression analysis Table 4, the predictors of cfPWV were duration of diabetes, systolic BP, triglyceride and FGF21. Then, the cutoff value for FGF21 for subclinical atherosclerosis risk was calculated and analyzed by Receiver operating characteristic (ROC) curve analysis and 95% confidence interval. In this study, the area under the curve (AUC) was found statistically significant (AUC = 0.715, 95% CI: 0.614–0.816, P < 0.001) in the total sample Fig. 2. As an optimal cutoff point, high risk FGF21 value of 184 pg/mL was determined with a 66.7% sensitivity and 66.7% specificity.

Table 3
Logistic regression analysis for predictors of subclinical atherosclerosis in DM.

Parameters	B	SE	P	OR	95% CI for OR
Dyslipidemia	2.020	.586	.001	7.539	2.390–23.776
Hypertension	1.676	.595	.005	5.344	1.666–17.146
FGF21	.012	.005	.023	1.012	1.002–1.023
Adiponectin	-.309	.117	.008	.734	.584–.923

SE, standard error; OR, odds ratio; CI, confidence interval. Variables included in the model were smoking, family history of cardiovascular disease, age, WC, duration of diabetes, FPG, eGFR, dyslipidemia, hypertension, adiponectin and FGF21.

4. Discussion

In the current study we demonstrated that diabetic patients with subclinical atherosclerosis have higher FGF21 than those without. There was a strongly positive significant correlation between fasting FGF21 and CIMT in the total sample (r = 0.452, p < 0.001) and in men and women groups. This correlation was preserved even after adjustment for age, BMI, WC and duration of diabetes (r = 0.328, p < 0.001). This agrees with previous study compared normoglycemic subjects and type 2 diabetes which found that FGF21 levels were significantly elevated in diabetes patients with carotid artery plaques [13]. Xiao et al. [14] in another study of 212 newly diagnosed subjects with type 2 diabetes found that elevated FGF21 serum levels were correlated positively with CIMT and iliac IMT, especially in women. Chow et al. [11] in a larger study of 670 subjects found this association is also gender-specific, they reported positive correlation of FGF21 with subclinical atherosclerosis, as measured by CIMT, only in women but not in men.

Lee et al. [20] in a large cohort of 3528 participants found that serum FGF21 level is an independent predictor of incident coronary heart disease in T2DM. Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial is the largest study which studied the role of FGF21 as a CVD risk marker in 9697 patients with type 2 diabetes [21]. The study found that serum FGF21 levels added to a risk prediction model which includes traditional cardiovascular risk factors and provided a better indication of the risk of a CVD event compared to the traditional model. More recently, a meta-analysis found that FGF21 not only predicts the incidence of coronary artery disease but also the risks of metabolic syndrome, diabetes mellitus and renal progression in diabetes [22].

The mechanism of elevated FGF21 in T2DM patients still under study. Generally, FGF21 is considered a novel metabolic regulator linked with various human diseases including type 2 diabetes, obesity, metabolic syndrome and atherosclerosis [23]. It was proposed that this paradoxically elevated level of FGF21 is attributed to FGF21 resistance or a compensatory mechanism to underlying metabolic stress, mainly a defense mechanism against atherosclerosis [14,24]. In cultured rat cardiac microvascular endothelial cells, FGF21 mRNA expression were elevated when these cells were cultured in atherosclerosis-like condition [25]. So, it was proposed that FGF21 might be secreted by endothelial cells in response to stress, and its elevated levels may be a signal of endothelial cell injury. In another study on mouse model, cardiac FGF21 expression was increased and significantly reduced cardiac cell apoptosis [26]. Moreover, a study on mice found that FGF21 deficiency make them more susceptible to hypercholesterolemia and atherosclerotic plaque formation and premature death [27]. Depending on these observations, this increase in serum FGF21 levels in subjects with diabetes or atherosclerosis could be a protective response to the vascular injury or inflammation in atherosclerosis.

FGF21 has the potential to be considered a biomarker for CVDs as it associated with coronary heart disease, myocardial ischemia, cardiac hypertrophy, and diabetic cardiomyopathy [10]. Clinical studies have observed an increased circulating level of FGF21 in atherosclerotic patients or those individuals who are at the high risk of developing this disease [11,12]. FIELD study showed that elevated baseline plasma FGF21 levels were associated with higher

Table 4
Stepwise multiple regression analysis for independent predictors of log transformed cfPWV in diabetes mellitus.

Model	Unstandardized Coefficients		Standardized Coefficients	t	*P*	95.0% CI
	B	SE	Beta			
Systolic BP	.003	.002	.172	2.210	.029	.000–.007
Duration of diabetes	.014	.005	.243	3.065	.003	.005–.024
FGF21 pg/mL	.001	.000	.276	3.437	.001	.000–.002
Triglyceride	.001	.000	.300	3.886	.000	.001–.002

CI, confidence interval; SE, standard error; Adjusted R^2 was 37.1% for the regression model.

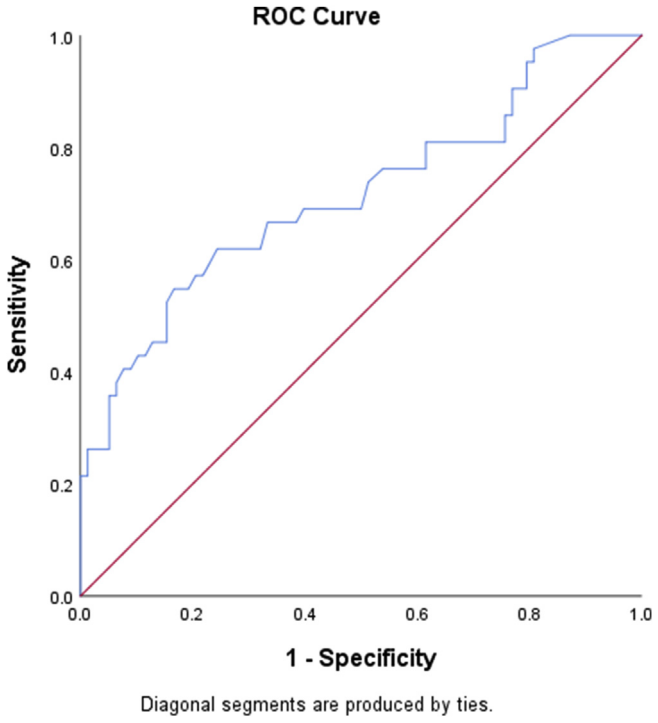

Fig. 2. ROC curve analysis for cutoff high-risk FGF21 level.

risk of cardiovascular events in T2DM patients over 5 years follow-up [28].Another study also suggested that the elevated FGF21 in the female patients with lower extremities atherosclerotic disease (LEAD) represent a compensatory mechanism, by which the system is attempting to protect against atherosclerosis [29].

Regarding association of FGF21 and ABI, we found significant negative association between FGF21 and ABI in the total sample ($P < 0.001$) and in women ($p = 0.002$) but not in men. Zhang et al. [29] reported that serum levels of FGF21 in female patients with lower limbs atherosclerosis were significantly higher than non-atherosclerotic patients, while no difference in male patients with or without LEAD. In contrast, Miyazaki et al. [30] reported that FGF-21 levels were low in patients with peripheral arterial disease. Recently, Li et al. [31] suggest a U-shaped relationship between FGF-21 levels and mortality as they found that both the highest and lowest FGF-21 levels were associated with an increased risk of cardiovascular mortality. They suggested that a very low FGF-21 level itself may be associated with diabetic complications and also a relatively elevated FGF-21 level may be a compensatory increase to protect against microvascular complication. Depending on our results and these studies we confirm that FGF21 could be used as a biomarker for subclinical atherosclerosis, particularly in patients with cardiovascular risk factors such as diabetes.

Interestingly, we reported for the first time the significant positive association of FGF21 with arterial stiffness in diabetes as it measured by cfPWV, the gold standard measures of arterial stiffness. In stepwise multiple regression analysis, FGF21 was found to be a predictor of cfPWV and arterial stiffness ($p = 0.001$). The role of FGF21 in arterial stiffness needs to be investigated in further studies with different population with and without cardiometabolic risk factors. Yang et al. [32] reported that FGF21 levels associated with arterial stiffness, as measured by baPWV and both significantly decreased after 3 months of the combined exercise in obese women.

The relation between FGF21 and anthropometric measures was studied extensively in different population. Zhang et al. [33] has demonstrated a strong positive association of serum FGF21 levels with age, BMI and waist circumference. Lin et al. [12] found that CHD subjects with diabetes had significantly higher serum FGF-21 levels than those of CHD subjects without diabetes ($P = 0.034$). They also reported a significant positive correlation of serum FGF-21 levels with BMI. In contrast, Lee et al. [34] reported that FGF21 have no correlation with BMI or WC. Li et al. [35] also reported lack of significant correlation between serum FGF21 and BMI or WHR. In the current study we found positive significant correlations of fasting FGF21 with WC and BMI in men only ($P = 0.010$ and $P = 0.003$ respectively).

With lot of controversies, the relation between FGF21 with duration of diabetes, FPG, HbA1c and Insulin was studied in different population. It was reported that serum FGF21 demonstrated a strong positive association with fasting insulin and HOMA-IR in obesity and diabetes including newly diagnosed and treated diabetes [33]. Another study reported that FGF21 was correlated with insulin, HOMA-IR, but not with FPG or HbA1c [34]. Lin et al. [12] found that serum FGF-21 levels correlated with fasting glucose and fasting insulin after adjustment for BMI. In the current study we found positive significant correlations between FGF21 and FPG and duration of diabetes in men ($P = 0.014$ and $P = 0.003$) and with FPG ($P = 0.005$) and HbA1c ($P = 0.001$) in women with no correlation with insulin in both sexes. This discrepancy in the results could be due to the effect of antidiabetic therapy.

Zhang et al. [29] found that FGF21 was correlated with FPG and HbA1c only in females while no correlation between FGF21 and duration of diabetes. In contrast, Li et al. [35] reported absence of significant correlation between serum FGF21 and FPG, HbA1c, fasting insulin or HOMA-IR in T2DM. Matuszek et al. [36] also reported lack of significant correlations between FGF-21 and insulin or HOMA-IR. Despite these controversies, the result of our study and previous reports support the hypothesis of compensatory response of FGF21 to facilitate glucose uptake that is dampened by insulin resistance.

The correlations of fasting FGF2 level with TG and HDL in T2DM patients was in line with previous study [29]. It is noticeable that long term Fenofibrate treatment could increase serum FGF21 levels; [37] however, in this study, we exclude subjects on such treatment. Zhang et al. [29] found that FGF21 was correlated with HDL, LDL only in females while correlated with TG in both sex and

no correlation between FGF21 total cholesterol. They conclude that these gender difference may be due to the difference of estrogen levels. Another study by Zhang et al. [33] has demonstrated a strong positive association of serum FGF21 levels with triglycerides and negatively with HDL cholesterol.

This controversy in studies could be explained by the fact that the lipid profile could be affected by different factors including BMI, waist circumference, smoking, diet, exercise and drug use which varied from one study to other. Recently, preclinical and clinical trials reported that therapeutic injection of FGF21 could alleviate dyslipidemia in obese and diabetic animals and human patients [38]. The pharmacological effects of FGF21 analog [39] or the long-acting FGF21 molecule [40] has been used to treat obese human subjects with type 2 diabetes with improvement in their lipid profiles.

FGF21 and adiponectin are the two main hormones secreted from the liver and adipose tissues respectively. Adiponectin has protective role in initiation and progression of atherosclerosis through its anti-inflammatory and antiatherogenic effects. Serum adiponectin levels are decreased in obesity, type 2 diabetes and patients with coronary artery disease [41]. In animal models, a single injection or chronic use of recombinant FGF21 increased serum level of adiponectin [42]. Interestingly, a clinical trial showed that injection of FGF21 analogue, LY2405319, induced increase in serum adiponectin in obese/diabetic patients [39]. Hui et al. [42] in his review suggested that FGF21–adiponectin axis protects against a different cardio-metabolic disorders via mediating multi-organ communications, and is a promising target for therapeutic interventions of these chronic diseases.

Few clinical studies found negative correlation of FGF21 with serum adiponectin [33,36]. In the current study we found strong negative correlation between FGF21 and adiponectin in diabetic men ($P = 0.004$) and in women group ($P = 0.003$). These evidence of disturbed FGF21–adiponectin axis with increased FGF21 and decreased adiponectin in obesity and diabetes, could be explained by FGF21 resistance. Eto et al. [43] found no correlation between adiponectin and FGF21 but they didn't exclude patients on fenofibrate treatment which could increase serum adiponectin and FGF21. Likewise, a recent clinical study in patients with metabolic syndrome confirmed the lack of association between FGF21 and adiponectin but they exclude diabetic patients in that study [44].

5. Limitations

There were some limitations in our study. In addition to the relatively small sample size, our study population was composed of Egyptian patients presenting at a single hospital. This study was cross sectional, so we can't explain the pathophysiological effect of FGF21 on arterial stiffness and on atherosclerosis. Furthermore, our finding of the association between serum FGF21 and arterial stiffness needs to be confirmed in other population-based studies involving larger sample sizes and different ethnicities with and without diabetes mellitus. Further longitudinal studies and clinical trials are in need to understand FGF21 physiology and maximize its therapeutic potential.

6. Conclusion

In conclusion, our study demonstrated that serum FGF21 levels are significantly correlated with carotid intima media thickness and predict subclinical atherosclerosis in patients with type 2 diabetes. We have shown for the first time that serum FGF21 is correlated positively with cfPWV and arterial stiffness in diabetes patients. The role of FGF21 as a biomarker of arterial stiffness needs further investigations.

Author contributions

S.Y. and F.E. wrote the manuscript, S.Y. collect data; M.E. performed doppler ultrasonography and cfPWV measurement; F.E., M.A. and E.Y. were involved in supervision and critical revision of the manuscript; S.Y and E.Y. performed data analysis and interpretation; All authors read and approved the final manuscript.

Funding

This research received no external funding.

Conflicts of interest

None.

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