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ORIGINAL ARTICLE

Circulating levels of fibroblast growth factor 21 in early-stage diabetic kidney disease

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Abstract

Aims/purpose Fibroblast growth factor 21 (FGF21), a hepatoadipokine with pleiotropic metabolic regulatory actions, is emerging as a novel biomarker of progressive nephropathy. We sought to evaluate circulating FGF21 and its association with clinical and biochemical characteristics as well as the urinary albumin excretion (UAE) rates in a population of patients with type 2 diabetes (T2D) with or without microalbuminuria and their matched healthy controls.

Methods Cross-sectionally, 130 consecutive individuals comprising patients with T2D with ($n = 44$) or without ($n = 44$) microalbuminuria and their healthy controls ($n = 42$) were recruited for analysis. Various demographic, clinical and biochemical parameters were assessed.

Results Serum FGF21 levels were significantly elevated in patients with microalbuminuria [median (interquartile range, IQR): 269.50 (188.50) pg/mL] compared to their normoalbuminuric peers with T2D [median (IQR): 103.50 (75.75) pg/mL] and nondiabetic people [median (IQR): 99.00 (126.75) pg/mL]. While serum FGF21, diastolic blood pressure and duration of diabetes mellitus (DDM) were independently associated with microalbuminuria in the baseline logistic regression model, FGF21 and DDM emerged as significant correlates in the multivariate adjusted model (OR for FGF21 = 1.060, 95% CI = 1.011–1.110, $P < .016$).

Conclusions Serum FGF21 level is strongly associated with early-stage diabetic kidney disease in the high-risk population of patients with T2D (particularly with circulating FGF21 values rising above 181 pg/mL). The association of serum FGF21 with subclinical stages of diabetic nephropathy may unearth perspectives on early detection and prevention of the advanced stages of chronic diabetes microvascular complications through effective FGF21-targeted therapy.

Keywords Fibroblast growth factor 21 · Diabetic Kidney Disease microalbuminuria · Urinary albumin excretion · Early biomarker

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Introduction

Diabetes is the leading cause of end-stage renal disease (ESRD) worldwide [1]. Microalbuminuria is often cited as a sensitive early marker for diabetic kidney disease (DKD)

and is thought to precede the more detrimental events seen in advanced stages of diabetic nephropathy [1]. During the past decade, however, the prognostic significance of microalbuminuria has come under increasing scrutiny for its high variability, and low sensitivity and specificity in predicting DKD progression [2]. In an observational study, 20% of patients with type 2 diabetes (T2D) had estimated glomerular filtration rate (GFR) decline to $\leq$60 ml/min/1.73m^2 before or even without passing the stage of microalbuminuria. Currently, it has been known that the progression of diabetic nephropathy, if defined as deterioration in chronic kidney disease (CKD) staging, is not necessarily paralleled with the progression of urinary albumin excretion (UAE) to microalbuminuria or macroalbuminuria [3]. Fibroblast growth factor 21 (FGF21), a hepatoadipokine with pleiotropic metabolic regulatory actions, is emerging as a novel biomarker of progressive nephropathy [4–6]. A cohort study of 1136 Chinese patients with T2D found two landmark findings: (1) baseline FGF21 levels act as an independent predictor of the decline in renal function with baseline eGFR $\geq$60 ml/min/1.73m^2 and (2) baseline FGF21 levels are an independent predictor of progression to micro- or macroalbuminuria and eGFR decline with a baseline eGFR <60 ml/min/1.73m^2 and normoalbuminuria [6]. Previous reports of the association between serum FGF21 with diabetic nephropathy [5] suggest that FGF21 may correlate with UAE at the preclinical stages of diabetic nephropathy when eGFR has not declined below 60 ml/min/1.73m^2 and/or microalbuminuria is not present.

As such and due to scarcity of studies to support this role for FGF21 as a potential early biomarker of DKD, we aimed to comparatively assess circulating FGF21 and UAE rates in a population of people with T2D with or without microalbuminuria and their matched healthy controls.

Patients and methods

Study design, population and protocol

The study population consisted of 130 consecutive individuals with type 2 diabetes (T2D) and their healthy controls. Patients with T2D were receiving oral antihyperglycemic agents and/or insulin and had regular follow-up visits at our diabetes day clinic (University Hospital of Vali-Asr, Tehran University of Medical Sciences). Patients with hypertension were taking either angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) and statins were used in patients with dyslipidemia. On the basis of pilot data, we estimated that 44 cases in each group (Group A: healthy controls; Group B: patients with T2D and normoalbuminuria, and Group C: patients with T2D and microalbuminuria) would provide 90% statistical power to detect a standardized difference of 0.70 in the mean FGF21 levels, assuming a two-sided type I error rate of 5%. Preliminary analysis of results revealed two control subjects with a borderline profile reminiscent of high-risk diabetic population, the data of whom was subsequently removed from the final analysis. Criteria for inclusion and exclusion of study participants are demonstrated in Table 1. Each patient received adequate information on study aims and protocol and signed informed consent forms prior to enrollment. The study protocol was approved by the local ethics committee and was conducted in accordance with the Helsinki Declaration.

Data collection

Waist circumference was taken at the end of normal expiration and in a horizontal plane, midway between the inferior margin of the ribs and superior border of the iliac crest, and was rounded to the nearest 0.1 cm. Body mass index (BMI; kg/m^2) was calculated according to the Quetelet equation. Blood pressure was measured after at least 5 min of rest in the sitting position, using a standard mercury sphygmomanometer. The average of two measurements made at least 5 min apart was used for analysis. FGF21 levels have been shown to exhibit a circadian rhythm and are subject to potential sampling bias [7]; therefore, venous blood samples were collected following an overnight 12-h fast at 7–8 AM for all participants. Fasting plasma glucose (FPG), glycated hemoglobin (HbA_{1c} %), total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoproteins cholesterol (HDL-C), blood urea nitrogen (BUN), uric acid, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (aP) were measured in a certified standard laboratory. Glucose measurements (intra- and interassay coefficients of variation 2.1 and 2.6%, respectively) were carried out using the glucose oxidase method. HbA_{1c} % was measured using high-performance liquid chromatography (Skylight Biotech, Akita, Japan). Serum triglycerides, total cholesterol, LDL-C and HDL-C concentrations were determined using enzymatic methods (Parsazmun, Karaj, Iran). Patients were informed to collect 24-h urine samples on three occasions within a few days after the visit. Gold standard method of 24-h urine collections helps avoid the false positive results associated with the use of albumin to creatinine ratio in a spot sample as the detection method [8]. The completeness of the collected sample was attested by measuring urinary creatinine excretion. A repeat measurement was requested if creatinine excretion levels were lower than 20 and 15 mg/kg per 24 h for men and women, respectively.

Table 1 Eligibility criteria for the study participants

Inclusion criteria
Type 2 diabetes with or without microalbuminuria
Regular follow-up visits at the diabetes day clinic of Vali-Asr Hospital
Exclusion criteria
Cigarette/tobacco smoking
Alcohol consumption
Obesity (body mass index ≥30 kg/m^2)
Evidence of impaired renal function (plasma creatinine >2 mg/dL and eGFR <60 ml/min/1.73m^2)
Presence of macroalbuminuria (urinary albumin excretion; UAE >300 mg/day)
Current urinary tract infection
Active bacterial and/or viral infection
Any evidence of fatty liver degenerative disease (nonalcoholic fatty liver disease and nonalcoholic steatohepatitis)
Use of drugs known to affect serum FGF21 levels (e.g., fenofibrate)
History of heart failure, ischemia in 12-lead electrocardiogram and other acute events (e.g., unstable and stable angina)
Any previous or current evidence of macrovascular disease (myocardial infarction, coronary artery disease, peripheral arterial disease, revascularization procedure or coronary artery bypass grafting and established atherosclerosis)

UAE was determined by calorimetric methods using commercial kits (ZiestChem Diagnostics, Tehran, Iran), with the average value obtained from three collections used for analysis. The Jaffe method was employed to assess serum concentrations of creatinine (Pars Azmun). Serum FGF21 concentrations were measured using a commercially available ELISA kit (Human Fibroblast growth factor 21 (FGF21/UNQ3115/PRO10196) ELISA kit, Cusabio Biotech, Wuhan, China); according to the instructions of the manufacturer. The reference range for detection was 15.6–1000 pg/mL with a sensitivity of 3.9 pg/mL. The intraassay and interassay coefficients of variability (CV) were <8 and <10%, respectively, with no cross-reactivity between human FGF21 and analogous FGF21 (e.g., FGF1, FGF2, FGF19, FGF23, etc.).

Definitions

Patients were stratified according to their UAE status as having either normoalbuminuria (UAE <30 mg/day) or microalbuminuria (UAE 30–299 mg/day) on at least two out of a total of three occasions (every other day during a week). Serial measurement of UAE by taking three urine collections per patient has the advantage of minimizing the effect of day-to-day variability of UAE. The diagnosis of diabetes was based upon the American Diabetes Association criteria [9]. With baseline creatinine concentrations available, the estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [10]: $\text{eGFR} = 144 \times (\text{Cr}/c)^{P} \times (0.993)^{\text{age}}$, where c (constant) = 0.7 in women and 0.9 in men; $P = -0.329$ in women with Cr ≤0.7 mg/dL, −1.209 in women with Cr >0.7 mg/dL, −0.411 in men with Cr ≤0.9 mg/dL and −1.209 in men with Cr >0.9 mg/dL. Using this formula, chronic kidney disease (CKD) was defined as eGFR <60 mL/min/1.73 m^2. Extensive details on the diagnosis of diabetic neuropathy are available elsewhere [11].

Statistical methodology

Data for this study were entered into the PASW software V 18.0 (IBM Corp., Armonk, NY, USA) for univariate and multivariate associations. Categorical variables were expressed as frequencies (%) and tested by Chi square (χ^2) test. Quantitative variables were expressed as median (interquartile range, IQR) since the normality of data was previously assessed and rejected by goodness-of-fit Shapiro–Wilk test. Between-group comparisons across the two (i.e., Group B and C) and three groups (i.e., Group A, B and C) was made using Mann–Whitney U and Kruskal–Wallis H tests, respectively. Univariate correlations were intended to test for the association between serum FGF21 and case mix variables as judged by Spearman rank correlation coefficients with the Bonferroni adjustment for multiple testing. A multivariable linear regression model was adopted to test for correlation among FGF21 as the dependent variable and UAE, eGFR and other Bonferroni-adjusted highly significant correlates (P value ≤.001) from the univariate analyses as well as clinically significant variables of age, sex, coexisting neuropathy and drug use. Abnormally distributed variables were formerly transformed using natural logarithm ($\log_e$) before entering the adjusted multiple linear regression model. Multivariate

binary logistic regression analysis was employed to assess the prediction of microalbuminuria (as binary dependent variable) by FGF21 in two models: model I: crude; model II: adjusted for age, gender and body composition indices (i.e., BMI and waist circumference), use of drugs (ACEi/ARB, statins and oral agents/insulin) and coexisting neuropathy. In both models, the results of regression are presented as odds ratios (OR) and 95% confidence intervals (95% CI). In each model, ORs (95% CIs) were calculated per one unit increase in the target variables to facilitate interpretation of regression coefficients [12].

Variables were considered in the multivariable logistic model that differed significantly between subjects with normoalbuminuria and microalbuminuria or if they were highly correlated with serum FGF21 in the univariate associations. For variables strongly related to each other, one representative variable was entered in the multivariate models to reduce the multicollinearity effect. We included the best fitting model with the most robust Hosmer–Lemeshow statistic after separate forward, backward and stepwise selection approaches. Sensitivity analysis of optimal cutoff value of serum FGF21 with maximum sensitivity/specificity pair for early DKD identification was run based on the probability receiver operating characteristic (ROC) curve. Only the diabetic subjects were selected for ROC analysis to emphasize the power of FGF21 as a predictive tool for the progression from normoalbuminuria to microalbuminuria. Two-sided statistical significance was defined at $P < .05$.

Results

The baseline characteristics of nondiabetic and diabetic individuals are summarized in Tables 2 and 3, respectively. The total prevalence of microalbuminuria in our series was 33.9%. Serum FGF21 levels were significantly elevated in patients with T2D and normoalbuminuria [median (IQR): 103.50 (75.75) pg/mL] compared with nondiabetic controls [median (IQR): 99.0 (126.75) pg/mL, $P < .001$]. The increase in serum FGF21 was also significant from the category of patients with T2D and normoalbuminuria to the group of patients with T2D and microalbuminuria [median (IQR): 269.50 (188.50) pg/mL, $P < .001$). Median FGF21 levels were significantly elevated in individuals receiving ARB and statin [246.00 (IQR: 250.00) for ARB use vs. 134.00 (IQR: 146.00) for ARB-free, $P = .004$; 233.00 (IQR: 248.25) for statin use vs. 135.00 (IQR: 146.75) for statin-free, $P = .009$]. However, subjects who were treated by insulin, oral agents or ACEi had comparable serum

Table 2 Baseline clinical characteristics of all individuals

	Control ($n = 42$)	T2D with normoalbuminuria ($n = 44$)	T2D with microalbuminuria ($n = 44$)	*P* value microalbuminuria vs. normoalbuminuria	*P* for trend
DDM (y)		8.11 ± 0.85	13.66 ± 0.93	<.001*	
Sex (men, %)	17 (40.5%)	21 (47.7%)	29 (65.9%)	.085	.051
Age (y)	52.52 ± 8.99	54.86 ± 7.54	56.98 ± 7.87	.118	.038*
BMI (kg/m^2)	26.42 ± 2.92	28.09 ± 3.16	29.34 ± 4.28	.194	<.001*
Waist circumference (cm)	89.73 ± 9.07	95.84 ± 8.58	99.14 ± 8.73	.076	<.001*
Systolic blood pressure (mm Hg)	120.00 (20.00)	130.00 (18.50)	130.00 (20.00)	.903	<.001*
Diastolic blood pressure (mm Hg)	75.00 (10.00)	80.00 (10.00)	80.00 (0)	.014*	.004*
Use of ACEi/ARB *n* (%)	1 (2.4%)	10 (22.7%)	26 (59.1%)	.001*	<.001*
Use of statins, *n* (%)	2 (4.8%)	12 (27.3%)	28 (63.8%)	.001*	<.001*
Hypoglycemic therapy					
Oral agents, *n* (%)		33 (75.0%)	40 (90.9%)	.047*	
Insulin, *n* (%)		6 (13.6%)	19 (43.2%)	.002*	
Oral agents + insulin, *n* (%) (%)		38 (86.4%)	43 (97.7%)	.049*	
Coexisting neuropathy		0	12 (27.2%)	<.001*	

Continuous variables are expressed as median (interquartile range)

DDM duration of diabetes mellitus, *BMI* body mass index, *ACEi* angiotensin-converting enzyme inhibitors, *ARB* angiotensin receptor blocker

* Significant correlations

Table 3 Biochemical characteristics and FGF21 levels in nondiabetic controls and in patients with T2D by categories of albuminuria

	Control ($n = 42$)	T2D with normoalbuminuria ($n = 44$)	T2D with microalbuminuria ($n = 44$)	P value normoalbuminuria vs. microalbuminuria	P for trend
Creatinine (mg/dL)	0.80 (0.21)	0.90 (0.30)	1.00 (0.30)	.028*	<.001*
BUN (mg/dL)	11.00 (3.25)	15.00 (8.75)	15.00 (7.00)	.913	<.001*
Uric acid (mg/dL)	4.10 (0.55)	4.50 (1.00)	5.15 (2.30)	.007*	<.001*
UAE (mg/day)		6.35 (5.50)	120.00 (95.75)	<.001*	
FPG (mg/dL)	88.00 (13.00)	152.50 (37.50)	148.50 (59.25)	.413	<.001*
HbA_{1c} (%)	4.85 (0.50)	6.80 (1.77)	8.00 (1.33)	<.001*	<.001*
(mmol/mol)	29.50	50.80	63.90		
Triglycerides (mg/dL)	98.00 (59.50)	130.50 (56.25)	160.50 (116.25)	.124	.001*
Total cholesterol (mg/dL)	179.50 (49.75)	147.50 (56.75)	160.00 (41.00)	.299	.020*
HDL-C (mg/dL)	50.00 (22.50)	42.50 (7.75)	39.00 (18.75)	.219	.004*
LDL-C (mg/dL)	100.00 (27.50)	82.50 (47.75)	83.50 (30.75)	.851	.009*
ASAT (units/L)	12.00 (9.00)	20.00 (7.75)	20.00 (11.00)	.661	<.001*
ALAT (units/L)	14.00 (6.25)	21.00 (15.00)	25.00 (19.00)	.099	<.001*
aP (units/L)	132.50 (69.50)	126.50 (56.00)	140.00 (60.00)	.837	.935
eGFR (ml/min/1.73m^2)	95.32 (19.48)	77.25 (40.39)	65.85 (38.58)	.021*	<.001*
FGF21 (pg/mL)	99.00 (126.75)	103.50 (75.75)	269.50 (188.50)	<.001*	<.001*

Continuous variables are expressed as median (interquartile range)

To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for BUN to millimoles per liter, multiply by 0.3571. To convert the values for FPG to millimoles per liter, multiply by 0.0555. To convert the values for triglycerides to millimoles per liter, multiply by 0.0113. To convert the values for total cholesterol, HDL-C, and LDL-C to micromoles per liter, multiply by 0.0259. To convert the values for ASAT, ALAT and aP to microkatal per liter, multiply by 0.0167

BUN blood urea nitrogen, *UAE* urinary albumin excretion, *FPG* fasting plasma glucose, *HbA_{1c}* glycated hemoglobin, *HDL-C* high-density lipoproteins cholesterol, *LDL-C* low-density lipoproteins cholesterol, *ALAT* alanine aminotransferase, *ASAT* aspartate aminotransferase, *aP* alkaline phosphatase, *eGFR* estimated glomerular filtration rate, *FGF21* fibroblast growth factor 21

* Significant correlations

FGF21 to their control medication-free counterparts ($P = .117$, .082 and .287, respectively).

The two groups of patients with T2D were comparable regarding body composition indices (BMI and waist circumference), BUN and some aspects of metabolic profile (systolic blood pressure, FPG and HDL-C). However, patients with normoalbuminuria and microalbuminuria (Groups B and C, respectively) were significantly different in terms of the duration of diabetes mellitus (DDM), use of different sets of medications, creatinine, serum uric acid, HbA_{1c} and eGFR (Tables 2 and 3).

Serum FGF21 levels were positively correlated with DDM ($r = .471$, $P < .001$), body composition indices [BMI ($r = .193$, $P = .027$) and waist circumference ($r = .224$, $P = .010$)], kidney function [creatinine ($r = .294$, $P = .001$), BUN ($r = .214$, $P = .014$) and serum uric acid ($r = .317$, $P < .001$)], glucose metabolism indices [FPG ($r = .352$, $P < .001$) and HbA_{1c} ($r = .451$, $P < .001$)], dyslipidemia (total cholesterol, LDL-C and triglycerides), liver function [ALAT ($r = .335$, $P < .001$) and ASAT ($r = .247$, $P = .005$)] and UAE ($r = .670$, $P < .001$) and negatively correlated with eGFR ($r = -.290$, $P = .001$). Associations with DDM, uric acid, UAE, FPG and ALAT retained their significant status after applying the Bonferroni correction for multiple testing (Table 4). No significant correlations were found concerning FGF21 and age, systolic or diastolic blood pressure, lipid profile status and aP. In separate analyses of the controls and patients with normoalbuminuria or microalbuminuria, the coefficients for these determinants were unreliable due to a fraction of patients available in each group, so we combined the groups for univariate and multivariate linear analyses. Highly correlated variables were selected for multivariate linear regression analysis (method: stepwise). In the multivariate model controlling for clinically significant variables of age, sex, coexisting

Table 4 Spearman's univariate correlations between FGF21 and markers of diabetic nephropathy

	r	*P* value
Age (y)	.125	.156
DDM (y)	.471	<.001*, ‡
BMI (kg/m^2)	.193	.027*
Waist circumference	.224	.010*
Systolic blood pressure (mmHg)	.007	.941
Diastolic blood pressure (mmHg)	.083	.348
Creatinine (mg/dL)	.294	.001*
BUN (mg/dL)	.214	.014*
Uric acid (mg/dL)	.317	<.001*, ‡
UAE$^{\alpha}$ (mg/day)	.670	<.001*, ‡
FPG (mg/dL)	.352	<.001*, ‡
HbA_{1c} (%)	.451	<.001*, ‡
Total cholesterol (mg/dL)	−.018	.840
HDL-C (mg/dL)	−.167	.058
LDL-C (mg/dL)	−.089	.313
ASAT$^{\alpha}$ (units/L)	.247	.005*
ALAT$^{\alpha}$ (units/L)	.334	<.001*, ‡
aP$^{\alpha}$ (units/L)	.014	.867
eGFR (ml/min/1.73m^2)	−.018	.001*

α Excluding control subjects. Abbreviations are indicated in Tables 2 and 3

* Significant correlations

‡ Correlations remaining significant after Bonferroni adjustment for multiple testing

neuropathy and drug use, UAE was the only variable associated with serum FGF21 values ($\beta = .646$, $P < .001$; per one standard deviation increase in Log-FGF21).

Clinical and biochemical variables that significantly differed between normoalbuminuria and microalbuminuria groups were examined with multivariate logistic regression models to predict microalbuminuria (Table 5). In the adjusted model, FGF21 emerged as the strongest correlate of microalbuminuria (OR for per one unit increase in serum FGF21 values = 1.060, 95% CI = 1.011–1.110, $P < .016$), followed by DDM (OR = 1.509, 95% CI = 1.010–2.256, $P = .045$) and diastolic blood pressure (OR = 1.233, 95% CI = 0.962–1.580, $P = .097$).

The cutoff threshold for serum FGF21 for the early diagnosis of DKD was calculated at 181 pg/mL with a sensitivity of 88.6% and specificity of 86.4% (Tables 6, 7). The same cutoff point was used in the ensuing multivariate adjusted model (Table 7). The ROC curve for accuracy of FGF21 in diagnosing early DKD is plotted in Fig. 1, with the criterion FGF21 value of 181 pg/mL having a positive predictive value (PPV) and negative predictive value (NPV) of 86.7 and 88.4%, respectively. Patients with serum FGF21 ≥181 pg/mL held a 49.4-fold increased risk of having entered the early stages of diabetic nephropathy. In the multivariate model, the adjusted odds ratio increased to 157.5 with a sensitivity of 93.2% and specificity of 92.3% ($P < .001$).

Discussion

While previous studies largely investigated the association of FGF21 with different stages of diabetic nephropathy [5, 6], we focused on this association among patients with T2D patients and microalbuminuria. Serum FGF21 showed a marked increase from the control group to T2D patients

Table 5 Predictors of microalbuminuria in the multivariate logistic regression analysis

	OR (95% CI)	Wald χ^2	*P* value
Model I; $R^2 = 0.806$			
DDM (y)	1.224 (1.018–1.472)	4.621	.032*
Diastolic blood pressure (mm Hg)	1.151 (1.020–1.299)	5.178	.023*
Uric acid (mg/dL)	0.858 (0.294–2.500)	0.079	.779
HbA1c (%)	0.947 (0.545–1.647)	0.037	.847
eGFR (ml/min/1.73m^2)	1.000 (0.955–1.047)	0.000	.990
FGF21 (pg/mL)	1.039 (1.015–1.063)	10.076	.002*
Model II; $R^2 = 0.906$			
DDM (y)	1.509 (1.010–2.256)	4.029	.045*
Diastolic blood pressure (mm Hg)	1.233 (0.962–1.580)	2.748	.097
Uric acid (mg/dL)	0.229 (0.015–3.483)	1.125	.289
HbA1c (%)	1.037 (0.257–4.174)	0.003	.960
eGFR (ml/min/1.73m^2)	1.008 (0.907–1.120)	0.021	.885
FGF21 (pg/mL)	1.060 (1.011–1.110)	5.829	.016*

Model I: crude; model II: adjusted by sex, age, body composition indices (BMI and waist circumference), use of drugs (ACEi/ARB, statins and oral agents/insulin) and coexisting neuropathy

* Significant correlations

Table 6 Cutoff value of serum FGF21 with sensitivity and specificity for prediction of early-stage DKD

Predictor	Cutoff level for early DKD (T2D with microalbuminuria)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Positive predictive value (%)	AUROC (95% CI)	*P* value
FGF21 (pg/mL)	≥181	88.6	86.4	86.7	88.4	0.942	<.001

AUROC area under the receiver operating characteristic curve, *CI* confidence interval

Table 7 Baseline and multivariate adjusted models for the analysis of serum FGF21 and early-stage DKD

Model	Predictor	Adjusted odds ratio (95% CI)	Sensitivity (%)	Specificity (%)	*P* value
Baseline	FGF21 (pg/mL)		88.6	86.4	<.001
	<181	1			
	≥181	49.4 (13.9–175.6)			
Multivariate adjusted	FGF21 (pg/mL)				
	<181	1	93.2	92.3	<.001
	≥181	157.5 (10.9–2269.9)			

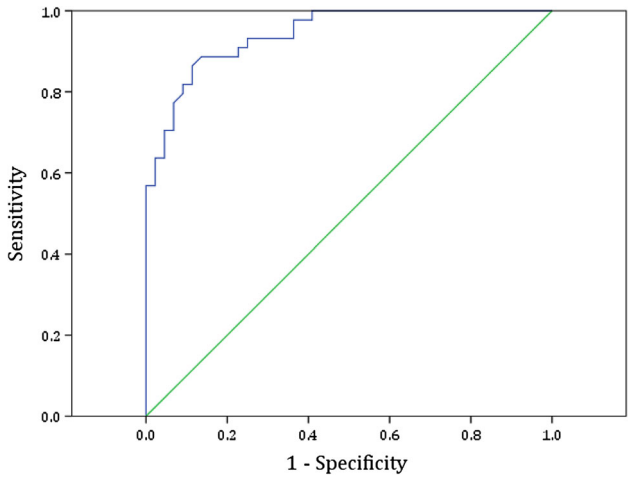

Fig. 1 ROC curve of the accuracy of serum FGF21 in predicting early-stage DKD. (area under the receiver operating characteristic curve = 0.942; see Table 6)

with microalbuminuria, with a substantial proportion of difference lying between normoalbuminuric and microalbuminuric patients. In the multivariable adjusted model, serum FGF21, and DDM were found to independently predict microalbuminuria in T2D patients. A high sensitivity/specificity pair for the calculated cutoff of 181 pg/mL supported the diagnostic credentials of FGF21 for early-stage DKD.

A recent Chinese study [5] reflected on serum FGF21 concentrations in T2D patients with different stages of diabetic nephropathy. The authors found significantly higher FGF21 concentrations in T2D patients with nephropathy compared to healthy controls. Their group of T2D patients with microalbuminuria had a higher median FGF21 concentration compared with the same group of our study, but our patients with microalbuminuria still had significantly lower FGF21 levels than their healthy controls [5]. Diverse methodology (i.e., ELISA kits) used to assess serum FGF21 is one of the potential reasons for this observation. Our patients with T2D and microalbuminuria had a longer duration of disease compared to this group in the study by Jian et al. [5]. Because insulin is a major upregulator of FGF21 synthesis in the body [13], increased insulin resistance and diminished insulin production associated with longer DDM could be another contributing factor to the difference in FGF21 concentrations between the two studies.

FGF21 was a strong and independent correlate of microalbuminuria. A clear understanding of the pathophysiology involved in the association between FGF21 and microalbuminuria merits further investigations. An important theory concerns the compensatory rise of serum FGF21 in response to the complicated metabolic derangement during the early stages of diabetic nephropathy. We have previously demonstrated the independent association of metabolic syndrome and microalbuminuria in patients with T2D [14].

Long-term diabetes (i.e., increasing DDM) and diastolic blood pressure were other independent correlates of microalbuminuria. These finding endorse DDM as the main contributing factor for the development of microalbuminuria in T2D rather than HbA_{1c} levels. While circulating HbA_{1c} is a reliable indicator of short-term uncontrolled diabetes, prolonged exposure to hyperglycemia-induced accumulation of advanced glycosylation end products are required for the progression from normoalbuminuria to microalbuminuria [15].

As such, sophisticated indicators (e.g., intrapersonal HbA_{1c} variability [16]) may prove to be more conclusive correlates of HbA_{1c} with microalbuminuria rather than the incidence mean HbA_{1c} values.

We extend the literature by demonstrating significant associations between the parameters of renal function (eGFR, BUN, creatinine and serum uric acid) and serum FGF21 among our patients with normoalbuminuria and microalbuminuria. Previously, this association was only reported among patients with diabetic nephropathy who had entered the clinical stage of macroalbuminuria [5]. Correlations of parameters of renal injury with raised FGF21 levels in normoalbuminuria and microalbuminuria are interesting, because the kidney profile in these subclinical phases is comparable to that of normal subjects. This finding understandably increases the possibility of an FGF21 altercation to cause renal damage; however, we have little evidence to support such a hypothesis. Experimental studies in rodent knockdown diabetics have revealed impaired local FGF21 expression in the kidney. In addition, higher expressions of FGF21 were observed in the renal and liver injured tissues; exogenous administration of FGF21 resulted in decreased renal apoptosis, regressed level of diabetes-induced renal inflammation, oxidative stress and fibrosis [2]. These observations may suggest a pathogenic role of FGF21 on the diabetes-induced progression of nephropathy.

Association of circulating FGF21 with hepatic enzymes has not previously been investigated in the high-risk diabetic population. Significant correlations of ASAT and ALAT with FGF21 in the univariate analysis did not remain significant following the adjustment in a multivariate model (Tables 4, 5). This is consistent with the reported higher titers of hepatic enzymes and increasing circulating FGF21 in longitudinal studies of the normal population [17], obesity [18], metabolic syndrome and nonalcoholic fatty liver disease (NAFLD) [19].

Univariate associations of ASAT, ALAT, aP and gamma-glutamyl transferase (γGT) in a survey of 670 nondiabetics was only significant for γGT in the multivariate adjusted model [20]. Circulating FGF21 has been shown to increase, possibly as a response to hepatic FGF21 resistance, in metabolic liver disease (e.g., NAFLD [21] and nonalcoholic steatohepatitis). Since AST, ALT and aP serve up as surrogate markers of fatty liver, an association between common hepatic enzymes with FGF21 is possible.

Following the correlation between the clinical indices of prolonged and uncontrolled diabetes and diabetic nephropathy with serum FGF21, we evaluated this marker with reference to its role in the clinical prediction of early DKD. We demonstrated that higher values of circulating FGF21 have more sensitivity rather than specificity for prediction of early-stage DKD. Nevertheless, our study is the first to address the association of serum FGF21 with the presence of early-stage DKD by using a high-yield cutoff and with a robust PPV and NPV. Subjects with the FGF21 above the clinical utility cutoff value of 181 pg/mL had a 157.5-fold increased chance of having entered early-stage DKD in the adjusted model. The exponential growth of UAE around the cut point for FGF21 is unequivocally confirmed by the scatter plot (graph not shown), to the extent that there is no subject with microalbuminuria with serum FGF21 values <108 pg/mL.

Positive correlation of FGF21 with parameters of body composition (BMI and waist circumference), FPG and HbA_{1c} in our study fits well with previous reports [5]. Despite the lack of an association between FGF21 with some facets of metabolic syndrome in our study (i.e., HDL-C and systolic or diastolic blood pressure) and other reports [20, 22], FGF21 has a wide array of metabolic characteristics and is expected to increase during the metabolic disturbance of the later stages of diabetic nephropathy [4].

Bobbert el al. in their follow-up study found that although FGF21 independently predicts metabolic syndrome and T2D, its blood levels is not correlated with lipid profile status, including HDL-C as a defining component of metabolic syndrome [22].

Considering the extensive regulatory involvement of FGF21 on carbohydrate and lipid metabolism and the link with diabetic nephropathy, FGF21-targeted treatment is a promising venue for research. FGF21 is capable of inducing both insulin-dependent and -independent glucose lowering actions through increasing GLUT1 expression and hyperactivity of WAT/BAT [23]. Insulin upregulation of SREBP1C to promote lipid synthesis in the liver is counteracted by FGF21-mediated programming of PPARGC1A to balance the triglyceride storage through enhancement of lipid oxidation [23]. Due to these additive (e.g., glucose uptake) and opposite (e.g., lipogenesis/lipid oxidation) behavior, a dual insulin/FGF21 therapy might be an exciting approach [23] to manage several obesity-related metabolic disorders, namely T2D and its complications (e.g., DKD).

There are several limitations to consider. First, the cross-sectional design draws no inference of causality on whether the raised FGF21 levels precede renal damage in diabetic nephropathy. Second, there are relatively small numbers of patients included in this study. Third, the measurement of plasma insulin, although potentially complicated by insulin therapy, could have played a defining role in elucidating the mechanistic basis of the association between FGF21 and microalbuminuria. Refractory insulin resistance and the accompanying hyperinsulinemia as the chief regulators of indigenous FGF21 production are the archetypal features of metabolic syndrome and are equally capable of inducing endothelial dysfunction.

Through fueling a diverse set of mechanisms including increasing the availability of endothelin-1, and changing intracellular calcium and magnesium metabolism [24, 25], this metabolic disturbance may result in increased vascular permeability and development/progression of microalbuminuria. Similarly, different rates of ACEi/ARB/oral agent medication use among the subset of T2D patients with or without microalbuminuria might potentially affect total FGF21 levels among these categories of patients, even though individuals receiving drugs that are known to alter serum FGF21 levels (e.g., fenofibrate) were excluded at baseline. Despite these suggestions, independent association of serum FGF21 and DKD was carefully adjusted for the presence of baseline demographic, clinical and biochemical factors, including the use of insulin/oral agents/ACEi/ARB. Fourth, calculating eGFR instead of gold-standard urinary iothalamate clearance [26] may have introduced measurement bias in our study. Finally, the use of UAE and its categorization into normoalbuminuria and microalbuminuria was intended to flag early DKD rather than the gold standard renal biopsy. However, decreased kidney function is detected also among patients with normoalbuminuria [27].

Not all DKD patients, in one study only 49% [28], would ever test positive for microalbuminuria. As demonstrated in chronic hemodialysis subjects [29] and community-dwelling participants with CKD [30], impaired renal function contributes significantly to the elevation of circulatory FGF21. However, we accounted for the inability of kidney to clear out FGF21 by excluding CKD patients (eGFR <60 ml/min/1.73m^2) from this study. Future studies should confine the association between FGF21 and UAE to subjects with normoalbuminuria, thus eliminating the influence of deteriorating renal function on this association.

In conclusion, higher levels of serum FGF21 in microalbuminuric than in normoalbuminuric patients suggests the association of circulating FGF21 with early-stage DKD, albeit that the current study offers little in terms of the underlying pathophysiology. Serum FGF21 level is strongly associated with microalbuminuria in the high-risk population of T2D. Future long-term follow-up studies are required to evaluate if serum FGF21 level is a true predictor of development, progression or death in DKD patients. However, it should be noted that the use of FGF21 outside of the research setting will require more research to elucidate if FGF21 is indeed a better predictor of diabetic nephropathy than a decline in eGFR and/or microalbuminuria. The association of FGF21 with preclinical stages of diabetic nephropathy may unearth perspectives on the early detection and prevention of the latter stages of the complication.

Our proposed diagnostic cutoff for serum FGF21, 181 pg/mL is subject to rigorous examination in large-scale prospective surveys to confirm if it is a true reflection of FGF21-related increased risk for early-stage DKD.

Compliance with ethical standards

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Conflict of interest Alireza Esteghamati declares that he has no conflict of interest. Amirhossein Khandan declares that he has no conflict of interest. Alireza Momeni declares that he has no conflict of interest. Aram Behdadnia declares that she has no conflict of interest. Alireza Ghajar declares that he has no conflict of interest. Mohammad Sadegh Nikdad declares that he has no conflict of interest. Sina Noshad declares that he has no conflict of interest. Manouchehr Nakhjavani declares that he has no conflict of interest. Mohsen Afarideh declares that he has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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