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EVALUATION OF CIRCULATING FIBROBLAST GROWTH FACTOR 21 AND FETUIN-A LEVELS IN TYPE 2 DIABETIC PATIENTS WITH NEPHROPATHY AND THEIR RELATIONS TO INSULIN RESISTANCE

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ABSTRACT

Early detection of the risk factors of diabetic nephropathy before advanced renal damage remains a major challenge, so biomarkers related to diabetic nephropathy are required. Our aim was to assess the levels of fibroblast growth factor 21 (FGF21) and fetuin-A as an early detector risk factors of nephropathy progression and also to explore the relationship between risk markers and glycemic control (HbA1c) on the one hand and lipid profile, insulin resistance, microalbuminuria and renal function tests on the other hand. A cross-sectional study with 200 participants. Each studied group included 50 subjects, conducted as group I: healthy control subjects were matched for age and gender as patients groups; group II: T2DM with normoalbuminuria [ACR (albumin/creatinine ratio) <30 mg/g creatinine]; group III: T2DM with microalbuminuria [ACR=30-300 mg/g creatinine]; group IV: T2DM with macroalbuminuria [ACR>300 mg/g creatinine]. Serum FGF21 and fetuin-A levels were measured using enzyme-linked immunosorbent assay. The plasma glucose, insulin, homeostasis model for the assessment of insulin resistance (HOMA-IR), HbA1c, lipids profile, urea, and creatinine levels were also measured. Plasma glucose, insulin, HbA1c levels and HOMA-IR were significantly elevated in T2DM groups as compared to the control group. These parameters represented pronounced increases in group III and IV as compared to group II. Significant elevation of total cholesterol, triacylglycerol as well as LDL-C and a significant reduction of HDL-C were reported in diabetic nephropathy as compared to groups I and II. The dramatic significant increase in FGF21 and fetuin-A levels were observed in diabetic patients with micro- and macroalbuminuria with a significant increase in all kidney function compared to those with a normoalbuminuric group. Elevated FGF21 and fetuin-A levels were parallel with the levels of diabetic biomarkers. FGF21 and fetuin-A were found to correlate positively with systolic blood pressure, glucose, HbA1c, HOMA-IR, cholesterol, triacylglycerol, LDL-C and kidney function tests in diabetic nephropathy groups. Serum FGF21 and fetuin-A levels are strongly associated with insulin resistance in T2DM with nephropathy and could be promising useful biomarkers for predicting nephropathy progression, especially at early stages of diabetic nephropathy. Furthermore, they may serve as a tool to monitor the impact of prevention and intervention on renal damage.

KEYWORDS: *Type 2 Diabetes Mellitus, Nephropathy, Fibroblast Growth Factor 21, Fetuin-A, Insulin Resistance.*

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a truly global affliction caused resistance to insulin action in multiple tissues, accompanied by failure of the pancreatic β-cells to compensate sufficiently by increased insulin secretion[1]. Diabetic complications are still a major concern since they constitute the main cause of morbidity and mortality in these patients. The most devastating complication is diabetic nephropathy (DN), which is associated with a markedly increased risk of end-stage renal failure, cardiovascular disease and premature death[2]. Diabetic nephropathy is one of the most common microvascular complication of diabetes mellitus[3]. It develops in approximately 40% of all type 2 diabetic patients, characterized by persistent albuminuria, elevated blood pressure and a progressive decline in kidney function leading towards end-stage renal disease (ESRD)[4] Early detection of renal injury leads to an early referral and consequently improved outcome. For quite a long time, the impaired renal function of patients with diabetic nephropathy is mainly reflected by laboratory detection of serum creatinine, and blood urea nitrogen, both of which are not sensitive enough to detect an early change of renal function[3]. As DN progresses, gradual increases in urinary albumin excretion and a decline in renal function may be observed, ultimately leading to end-stage renal disease[4]. Accordingly, microalbuminuria is considered to be an important prognostic marker for the early detection of DN[5]. However, it has been shown that many patients with diabetes may still develop DN, even if their urinary albumin levels are within the normal range. Moreover, it has also been suggested that albuminuria is not a good early marker, since it is only possible to observe microalbuminuria when significant damage to glomerular function has already occurred[6]. Therefore, more sensitive and specific biomarkers are required to detect DN at an earlier stage[5]. It has been demonstrated that renal injuries in DN are particularly heterogeneous and that almost all of the cellular elements in a diabetic kidney may be affected. In addition to glomerular dysfunction, tubulointerstitial damage may also be important in the pathogenesis and progression of DN[6]. Fibroblast growth factor 21 (FGF21) is a 210-amino acid peptide belonging to the FGF19 family[7]. In rodents, FGF21 mRNA is widely expressed in peripheral metabolic tissues, such as the liver, pancreas, skeletal muscle, and white adipose tissue[8]. FGF21 protein also has been detected in plasma[9], which suggests that it is secreted into systemic circulation and may act like a hormone. Indeed, FGF21 binds to FGF receptors and its coreceptor β-klotho on plasma membranes[10], activating Akt signaling pathways[11]. Prolonged fasting was found to increase hepatic FGF21 transcription in rodents, and this increased transcription was mediated by peroxisome proliferator activated receptor-α (PPARα)[12]. Elevated FGF21 levels during fasting appear to be important for metabolic adaptation to a low-energy state by inducing increased ketogenesis, lipolysis, and torpor-like behavior[13]. On the other hand, accumulated data demonstrate the beneficial effects of FGF21 on glucose and lipid metabolism[14]. FGF21 overexpressing mice were resistant to the development of diet-induced obesity[15]. In humans, morning fasting serum FGF21 concentrations were not significantly altered by short-term fasting but were increased by treatment with the PPARα ligand fenofibrate[16]. Circulating FGF21 concentrations also were elevated in patients with type 2 diabetes compared to non-diabetic individuals[17]. Moreover, fasting serum FGF21 levels were positively associated with parameters of obesity, such as body mass index (BMI) and waist to hip ratio (WHR)[18], although another study reported no such correlation[17]. However, the physiological roles of FGF21 in human metabolism remain obscure. Serum FGF21 levels have been shown to be increased in patients with impaired renal function. Patients undergoing chronic hemodialysis have elevated serum FGF21 levels, more than 15-fold that of controls[19] while serum creatinine and GFR are inversely related to circulating FGF21 levels in control subjects. FGF21 is eliminated by the kidneys and its level increases as the stage of chronic kidney disease progresses[20]. A potential biomarker of progression in diabetic nephropathy have been identified; fetuin-A also called α-Schmid Heremans glycoprotein, is a potent systemic calcification inhibitor[21]. Low level of fetuin-A is associated with cardiovascular mortality in patients on dialysis[22]. In addition, the low fetuin-A level has been linked to vascular calcification[23] and flow-limiting aortic stenosis[24]. Fetuin-A interacts with the insulin receptor tyrosine kinase and induces insulin resistance in rodents[25]. Stefan et al.[26] demonstrated in a prospective case-cohort study that elevated fetuin-A level is a dependent risk factor for developing diabetes. In addition to renal (dialysis) patients, several studies showed that high level of fetuin-A is associated with atherosclerosis and its manifestations in nonrenal patients[27]. A recent study has indicated that fetuin-A level decreases in uremic patients on hemodialysis in comparison with normal healthy controls. The low fetuin-A level in patients with chronic kidney failure is strongly associated with a higher cardiovascular mortality. In contrast, it was demonstrated that higher than normal level of serum fetuin-A in older populations is associated with incident diabetes, which is independent of other markers of insulin resistance. Furthermore, a higher fetuin-A level may be an independent risk marker in patients with cardiovascular disease [22]. There is no data available for the association of fetuin-A with parameters of microvascular disease in diabetes[28]. Notably, once nephropathy develops, about 20 – 40% inevitably progress to end-stage renal disease (ESRD) [29]. Therefore, the present study was undertaken to assess the levels of fibroblast growth factor 21 (FGF21) and fetuin-A as an early risk detector factors of nephropathy progression. This study was extended to explore the relationship between risk factors and glycemic control (HbA1c) on the one hand and lipid profile, HOMA-IR, microalbuminuria, ACR and renal function tests on the other hand.

MATERIALS AND METHODS

Materials

Patients

Our study included 200 subjects who were categorized into four groups. Type 2 DM was diagnosed according to the World Health Organization Expert Committee of diabetes mellitus[30]. Patients were divided according urinary albumin excretion which was measured by spot morning urine sample for albumin to creatinine ratio (ACR) into 3 groups diabetic with normoalbuminuria (ACR<30 mg/g creatinine), diabetic with microalbuminuria (ACR between 30-300 mg/g creatinine) and diabetic with macroalbuminuria (ACR > 300 mg/g creatinine). All subjects underwent full history taking and clinical examination. The control group included 50 subjects were matched for age and gender as patients groups and had no evidence of ischemic heart disease, as proven by history, and no clinical evidence of previous strokes or peripheral vascular disease.All cases and controls who participated in our study were fully informed of the aim of the study and gave written consent for their participation and their agreement that, provided that their anonymity is maintained. For all participant, complete history and comprehensive questionnaires were used to collect medical information, anthropometric measurements, and clinical examinations were performed. Body mass index (BMI) was calculated as weight in kilograms divided by square of the height in meters (Kg/m^2) [31]. In addition, waist circumferences (WC) were measured at the mid distance between the iliac crest and last rib margin at the level of symphysis pubis [32]. Blood pressure measurements were performed by trained technicians or nurses with a mercury sphygmomanometer and the first and fifth Korotkoff sounds were recorded to represent the systolic and diastolic pressures. Three measurements were obtained on each occasion, at 5-min intervals and averaged. Hypertension was defined as present if the systolic blood pressure (SBP) ≥ 140 mm Hg or diastolic blood pressure (DBP) ≥ 90 mm Hg, . Patients were invited to participate in the study when they met criteria for inclusion (diabetic nephropathy; microalbuminuria; and macroalbuminuria; type 2 diabetic patients were taking the same therapy). Patients with any history of smoking, alcohol habits, respiratory disorder, clinical or laboratory sing of liver disease, thyroid function impairments, and chronic inflammation were excluded.

Blood Sampling

Blood samples were collected after 12 h overnight fasting from healthy subjects and diabetic patients into three types of vacutainer tubes processed as follows: first vacutainer tube with potassium oxalate and sodium fluoride (gray cap) for assaying of plasma glucose at once. Second vacutainer tube with EDTA (lavender cap) without centrifugation (whole blood sample) for assaying HbA1c. Third vacutainer tube without additive (red cap), blood was centrifuged at 4000 rpm for 10 minutes. Sera were rapidly separated and subdivided into aliquots one of them to measure lipid profile (cholesterol, triacylglycerol, HDL-C & LDL-C), urea and creatinine on the same day as the blood was collected and the remaining aliquot sera were stored at -80ºC for insulin, FGF21 and Fetuin-A determinations. Hemolyzed samples were excluded. Fresh early morning urine sample was collected from each subject into a sterile container and used for determination of microalbumin and ACR.

Methods

Plasma glucose concentration was assayed by glucose oxidase method[33]. Blood HbA1c was measured using high-performance liquid chromatography (HPLC) (Variant™ II; Bio-Rad, Hercules, CA, USA)[34]. Serum insulin concentration was measured using commercially available enzyme- linked immunosorbent assay (ELISA) kits Immunospec (Catalog No. 29-072)[35]. The homeostasis model for the assessment of insulin resistance (HOMA-IR) was calculated as the fasting plasma glucose level (mmol/L) x fasting plasma insulin level (mU/L) divided by 22.5 [36,37]. Lipid profile parameters; Commercial kits based on different techniques, purchased from Bio Med, Egy-Chem were used for the determinations of serum total cholesterol[38], HDL-C[39] and triacylglycerol[40]. LDL-C was calculated according to Friedewald et al.,[41]. Serum urea and creatinine (Cr) levels were determined using an enzymatic colorimetric method[42,43]. Sampling, reagent delivery, mixing, processing, calculating and printing were full automatically performed by the Dimension® RxL Max Integrated chemistry system (SEIMENS instruments Inc, USA). The multiagent microalbumin assay (turbidimetric immunoassay) is used for the quantitative measurement of albumin in human urine on the ARCHITECT c8000TM System. The kit was provided by Abbott Diagnostics. It's found that it is more typical to compare the amount of albumin in the urine sample against its concentration of creatinine. This is termed the albumin-to-creatinine ratio "ACR" expressed in 'mg/g creatinine'. ACR was estimated by dividing the µalb. Concentration in mg/L over the urine creatinine concentration in g/L. ACR was thus expressed in mg/g creatinine. Human FGF21 enzyme-linked immunosorbent assay (ELISA) kits were obtained from BioVendor Laboratory Medicine (Modrice, Czech Republic). [45] Serum concentrations of fetuin-A were assessed by a commercially available ELISA (BioVendor Laboratory Medicine, Modrice, Czech Republic). Measured intra-assay and interassay variability coefficients were 10.2 and 10.0%, respectively[22].

STATISTICAL ANALYSIS

Data were statistically expressed as mean ± standard deviation (SD). Comparison of more than 2 variables was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. The correlations between various variables were calculated using Pearson correlation coefficient. A probability value (P) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS version 20 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA).

RESULTS

The clinical characteristics of the diabetic patients and healthy control group are listed in Table 1. Our results showed that there was no significant difference between all of the studied groups regarding age, BMI, and WC. The means of age and BMI for groups I, II and III and IV are illustrated in Table (1). The means of age were 55.0±6.19, 56.8±6.7, 55.5±5.9 and 55.4±6.0 years and that of BMI were 26.5±3.1, 27.1±1.4, 28.5±2.6 and 28.4±2.2 kg/m2 for groups I, II III, and IV, respectively. The ANOVA test showed no significant differences between the means of age and BMI among different groups (P >0.05) indicating that all studied groups were matched for age and BMI. This will give reliable comparable results for diabetic nephropathy. The mean values of diabetes duration in groups II, III & VI. There was an increasing trend in the duration of diabetes recording mean values of 4.4±3.9, 6.5±5.4, and 8.9±6.1 years in groups II, III and IV, respectively. However such increase was significant (P >0.05) (Table 1). We demonstrated a highly significant increase in systolic and diastolic blood pressure levels in diabetic nephropathy groups when compared to healthy control group (P < 0.0001).

Table 1
Characteristic features of studied groups

Variables	Group I = 50	Group II = 50	Group III = 50	Group IV = 50
Age (years)	55.0 ± 6.19	56.8 ± 6.7	55.5 ± 5.9	55.4 ± 6.0
Gender (M/F)	23/27	21/29	20/30	18/32
Duration of DM (years)	------	4.4± 3.9	6.5± 5.4[a,b]	8.9± 6.1[a,c]
BMI (kg/ m^2)	26.5 ± 3.1	27.1± 1.4	28.5± 2.6	28.4 ± 2.2
WC (cm)	83.3± 6.5	84.5± 5.9	85.7± 6.3	85.4± 6.8
Systolic BP (mmHg)	118.1 ± 5.9	125.7 ± 7.3	137.1 ± 6.7[a]	152.4 ± 6.4[a]
Diastolic BP (mm Hg)	76.6 ± 5.0	81.4 ± 6.2	87.1 ± 4.3[a,b]	92.7 ± 5.1[a]

Values are represented as mean ± SD. Group I (control), Group II (diabetic patients with normoalbuminuria), Group III (diabetic patients with microalbuminuria), Group IV (diabetic patients with macroalbuminuria). Abbreviations: n: number of subjects M/F: Male/Female, DM: Diabetes Mellitus, BMI: Body Mass Index, WC: waist circumferences, BP: Blood Pressure.
[a]p < 0.0001 Vs. CON, [b]p< 0.0001 versus group II, [c]p< 0.0001versus group III..

As illustrated in Table 2, Fasting plasma glucose, serum insulin, HOMA-IR and HbA1c%, showed significant increases (P<0.0001) in type 2 diabetic patients with normoalbuminuria, microalbuminuria, and pronounced increases in diabetic patients with macroalbuminuria compared to normal control subjects. Type 2 diabetic patients with microalbuminuria and macroalbuminuria showed significant increases (P <0.0001) in fasting plasma glucose, plasma insulin, HOMA-IR and HbA1c%values, compared to diabetic patients with normoalbuminuria.

Table 2
Fasting plasma glucose, HbA1c, plasma insulin levels and HOMA-IR scores of participants included in the different studied groups.

Variables	Group I	Group II	Group III	Group IV
Fasting plasma glucose (mmol/L)	5.02 ± 0.53	10.57± 1.21[a]	13.6 ± 1.73[a,b]	16.9 ± 1.95[a,b,c]
Serum insulin (mU/L)	7.6± 1.5	13.8 ± 2.4 a	14.8 ± 2.2[a,b]	18.6 ± 3.6[a,b,c]
HOMA-IR	1.69±0.37	6.62± 1.74 a	9.07 ± 2.40[a,b]	14.25 ± 4.29[a,b,c]
HbA1c ((%)	5.29± 0.56	8.94± 1.04[a]	9.15 ± 0.91[a,b]	11.68± 1.61[a,b,c]

Abbreviations: HOMA-IR: homeostasis model for the assessment of insulin resistance, HbA1c: glycosylated hemoglobin
[a]p < 0.0001 Vs. CON, [b]p < 0.0001 Vs. Group II, [,c]p < 0.0001 Vs. Group III.

Table (3) points out that the mean values of total cholesterol, triacylglycerol and LDL-C were significantly increased in the diabetic group with microalbuminuria (225.2± 12.3, 110.2± 10.9 and 164.3±14.3 mg/dL respectively), being more pronounced in macroalbuminuria group (278.5± 12.9, 161.2± 14.8 and 213.7±14.5 mg/dL respectively) when compared with normoalbuminuria (161.2± 18.5, 76.9± 10.92 and 105.7±15.9 mg/dL respectively) and control groups (154.3± 14.89, 69.6± 12.63 and 89.0±15.3 mg/dL respectively) at p < 0.0001. Also, there was a significant increase in total cholesterol, triacylglycerol and LDL-C in the diabetic group with normoalbuminuria compared to control group at p < 0.0001. While the mean serum value of HDL-C was significantly decreased in the diabetic group with microalbuminuria (38.9±5.9 mg/dL), being more pronounced decreased in macroalbuminuria group (32.5±4.6 mg/dL) when compared with normoalbuminuria (43.7±7.8 mg/L and control groups (51.1±5.7 mg/dL) at p< 0.0001. Also, there was a significant decrease of HDL-C in the diabetic group with normoalbuminuria compared to control group at p < 0.0001.

Table 3
*Lipid profile of participants included in
the different studied groups.*

Variables	Group I	Group II	Group III	Group IV
Total cholesterol (mg/dL)	154.3± 14.9	161.2± 18.5 [a]	225.2± 12.3 [a,b]	278.5± 12.9 [a,b]
Triacylglycerol (mg/dL)	69.6± 12.63	76.9± 11.92 [a]	110.2± 10.9 [a,b]	161.2± 14.8 [a,b]
HDL-C (mg/dL)	51.1±5.7	43.7±7.8 [a]	38.9±5.9 [a,b]	32.5±4.6 [a,b]
LDL-C (mg/dL)	89.0±15.3	105.7±15.9 [a]	164.3±14.3 [a,b]	213.7±14.5 [a,b]

Abbreviations: HDL-C: high-density lipoproteins-cholesterol, LDL-C: low-density lipoproteins-cholesterol
[a] p < 0.0001 Vs. CON, [b] p < 0.0001 Vs. Group II,

The mean values of serum urea, creatinine. urinary microalbumin and A/C ratio of diabetic patient with microalbuminuria were significantly increased (53.9±10.8 mg/dL, 1.35±0.36 mg/dL, 72.2±26.48 mg/L and 59.13±21.14 mg/g creanitine respectively), being more pronounced in macroalbuminuria group (63.97±11.26 mg/dL, 1.87±0.42 mg/dL, 741.1±65.31 mg/L and 598.7±92.62 mg/g creatinine respectively) when compared with normoalbuminuria (28.6±4.86 mg/dL, 0.83±0.21 mg/dL, 13.7±8.2 mg/L and 10.8±3.40 mg/g creatinine respectively) and control groups (26.9±5.49 mg/dL, 0.76±0.194 mg/dL, 12.3±5.23 mg/L and 9.8±3.81 mg/g creatinine respectively) at p<0.0001, but there was no significant difference between the diabetic group with normoalbuminuria and the control group (p > 0.05) (Table 4).

Table 4
*Levels of serum Urea. creatinine, urine microalbumin and
Urinary A/C ratio in the different studied groups.*

Variables	Group I	Group II	Group III	Group IV
ea (mg/dL)	26.9±5.49	28.6±4.86	53.9±10.87 [a,b]	63.97±11.26 [a,b,c]
Creatinine (mg/dL)	0.76±0.194	0.83±0.21	1.35±0.36 [a,b]	1.87±0.42 [a,b,c]
Urine microalbumin (mg/L)	12.3±5.23	13.7±8.2	72.2±26.48 [a,b]	741.1±65.31 [a,b,c]
UA/C ratio (mg/g creatinine)	9.8±3.81	10.8±3.40	59.13±21.14 [a,b]	598.7±92.62 [a,b,c]

Abbreviations: UA/C ratio: urinary albumin/creatinine ratio.
[a] p < 0.0001 Vs. CON, [b] p < 0.0001 Vs. Group II, [c] p < 0.0001 Vs. Group III.

Serum FGF21 and fetuin-A levels were significantly higher in type 2 diabetic patients with microalbuminuria (407.7±40.6 pg/mL, 291.3±20.5 µg/mL respectively), being more pronounced in macroalbuminuric type 2 diabetic patients (569.5±36.1 pg/mL, 386.7±32.9 pg/mL respectively) when compared to normoalbuminuric type 2 diabetic patients (335.1±43.5 pg/mL, 250.9±32.9 µg/mL respectively) and healthy control (150.3±26.5 pg/mL, 168.9±18.6 µg/mL respectively) at p<0.0001 (Table 5).

Table 5
*Levels of serum FGF21 and Fetuin-A in
the different studied groups.*

Variables	Group I	Group II	Group III	Group IV
Serum FGF21 (pg/mL)	150.3±26.5	167.1±29.5	407.7±40.6 [a,b]	569.5±36.1 [a,b,c]
Serum fetuin-A(µg/mL)	168.9±18.6	179.9±19.9	291.3±20.5 [a,b]	386.7±32.9 [a,b,c]

Abbreviations: FGF21: Fibroblast Growth Factor 21
[a] p < 0.0001 Vs. CON, [b] p < 0.0001 Vs. Group II, [c] p < 0.0001 Vs. Group III.

Pearson's correlation analyzes revealed the relationships between FGF21 and fetuin-A and the different parameters studied in diabetic patients type 2 with microalbuminuria and macroalbuminuria. Pearson's correlation showed that FGF21 and fetuin-A concentrations correlated significantly with fasting plasma glucose and HbA1c (r = 0.828 , P < 0.0001 - r = 0.874 , P < 0.003) for FGF21 and (r = 0.823 , P < 0.0001 - r = 0.897 , P < 0.0001) respectively for fetuin-A in diabetic patients type 2 with microalbuminuria . In the same patients varying degrees of significant correlations were recorded between FGF21 and fetuin-A and creatinine, urine microalbumin and U A/C ratio (r = 0.595 , P < 0.019 - r = 0.706, P < 0.003 - r = 0.797, P < 0.0001) for FGF21 and (r = 0.559 , P < 0.03 - r = 0.674 , P < 0.006 - r = 0.755, P < 0.001) respectively for fetuin-A. FGF21 and fetuin-A were correlated significantly with HOMA-IR (insulin resistance) (r = 0.861, P < 0.0001 - r = 0.855, P < 0.0001). Also the concentrations of FGF21 and fetuin-A were positive correlated with triglycerides, total cholesterol and LDL-C levels (r = 0,759 , P < 0.001 - r = 0.741, P < 0.002 - r = 0.559, P < 0.03) for FGF21 and (r = 0.724 , P < 0.002 - r = 0.763 , P < 0.001 - r = 0.640, P < 0.01) respectively for fetuin-A in diabetic patients type 2 with microalbuminuria. In type 2 diabetic patients with macroalbuminuia, FGF21 level was correlated significantly with fasting plasma glucose and HbA1c (r = 0.777 , P < 0.001 - r = 0.920, P < 0.0001) , with HOMA-IR (insulin resistance) (r = 0.829, P < 0.0001) and with UA/C ratio (r = 0.788, P < 0.0001), while the level of fetuin-A correlated statistically with triacylglycerol (r = 0.565, P < 0.028) and total cholesterol level (r = 0.645, P < 0.009). All significant correlations for the studied groups are summarized in table 6.

Table 6
Significant correlations between FGF21 and fetuin-A and other
biochemical parameters in nephropathy groups

Parameters	Diabetic nephropathy groups							
	Microalbuminuric group				Macroalbuminuric group			
	FGF21		Fetuin-A		FGF21		Fetuin-A	
	r	P	r	P	r	P	r	P
Glucose	0.828	0.0001**	0.823	0.0001**	0.777	0.001**	0.682	0.005**
Insulin	0.846	0.0001**	0.845	0.0001**	0.900	0.0001**	0.585	0.022*
HOMA-IR	0.861	0.0001**	0.855	0.0001**	0.829	0.0001**	0.649	0.009**
HbA1c	0.874	0.003**	0.897	0.0001**	0.920	0.0001**	0.625	0.013*
Total cholesterol	0.741	0.002**	0.763	0.0001**	0.916	0.0001**	0.645	0.009**
Triglycerides	0.759	0,001**	0.724	0.002**	0.962	0.0001**	0.565	0.028*
LDL-C	0.559	0.03*	0.640	0.01*	0.872	0.0001**	0.677	0.006**
Urea	0.740	0,002**	0.618	0.014*	0.854	0.0001**	0.663	0.007**
Creatinine	0.595	0.019*	0.559	0.03*	0.821	0.0001**	0.653	0.008**
Miroalbuminuria	0.706	0.003**	0.674	0.006**	0.838	0.0001**	0.660	0.007**
UA/C ratio	0.797	0.0001**	0.755	0.001**	0.788	0.0001**	0.756	0.001**
BMI	0.734	0.002**	0.730	0.002**	0.526	0.044*	0.526	0.044*
Systolic BP	0.724	0.001**	0.724	0.002**	0.579	0.24*	0.529	0.043*

r : Pearson's Correlation Coefficient.

DISCUSSION

Diabetic nephropathy is one of the most common microvascular complication of diabetes mellitus[3]. Identification of markers for early detection of diabetic nephropathy remains a challenge in disease management [46]. Thus, we assess FGF21 and Fetuin-A levels as risk factors and predictive early detector markers of nephropathy progression. Also, we explore the relationship between these risk factors and glycemic control (HbA1c) on the one hand and lipid profile, HOMA-IR, microalbuminuria, ACR and renal function tests on the other hand. In the present study no significant difference was observed between the ages of the studied groups. The duration of diabetes in the diabetic group with microalbuminuria was significantly increased, being more pronounced in macroalbuminuria group as compared to normoalbuminuria group. The levels of fasting plasma glucose and HbA1c in the diabetic group with microalbuminuria was significantly increased, being more pronounced in macroalbuminuria group as compared to normoalbuminuria and control groups. Higher levels of HbA1c were associated with increased risk for development of microangiopathy in diabetes. This may be due to the fact that HbA1c has a special affinity for oxygen thereby causing tissue anoxia and plays a role in the causation of micro and macroangiopathy[47]. Albuminuria was associated with poor glucose control (revealed by high HbA1c levels). Poor glycemic control may have a significant role in the progression of diabetic nephropathy in these patients [48]. Diabetics with poor glycemic control had higher microalbumin levels compared with those of diabetics with good glycemic control[47]. These results in agreement with the findings of Altan[49] . stated that HbA1c level in the patients with elevated 24 hours urinary albumin was significantly increased compared with those without microalbuminuria, with the highest level in the patients with significant renal insufficiency. The results of the present study are consistent with study of Wang et al.[50] and not consistent with Li et.al.[51]. In humans, circulating FGF21 levels increased but only after a 7 days fast[16]. No increase in FGF21 levels was observed after a 3 days fast in healthy women[19]. FGF21 has also induced the liver during the adaptive starvation response in mice[52]. The circulating FGF21 concentrations should be elevated with subjects with impaired glucose metabolismbecause of FGF21 resistance or do the increased levels represent a compensatory response to facilitate glucose uptake that is blunted by insulin resistance. The present study appears to support the latter mechanism because elevated circulating FGF21 levels were positively associated with HOMA-IR. Our results showed elevated levels of circulating fetuin-A in diabetic patients with normoalbuminuria compared to control group and in T2DM with micro- and macroalbuminuria groups compared with T2DM with normoalbuminuria are in accordance with Inoue et al.[53] who found that mean concentration of fetuin-A of diabetic patients were on average 21% higher than those of controls. Therefore increased fetuin-A levels provides a sound rationale to select fetuin-A as a target for treating or preventing diabetes-associated micro- or macrovascular complications. There was a positive association of fetuin-A with insulin resistance, this result was confirmed by Ishibashi et al.[54]. In this study, we observed that serum total cholesterol, triacylglycerol, and LDL-C were significantly higher in diabetic nephropathy groups compared with normoalbuminuria and normal control groups. On the other hand, the serum level of HDL-C was significantly decreased in diabetic nephropathy groups compared with normoalbuminuria and normal control groups. These results agree with reports that the role of lipotoxicity and oxidative stress in up-regulation of FGF21 synthesis[55]. Also these results in agreement with Bonnet and Cooper[56] who stated that diabetic nephropathy is associated with an altered lipid profile characterized by elevated triglyceride-rich lipoproteins even in the early stages of the renal disease. Apolipoproteins and lipoproteins increased at the stages of microalbuminuria and macroalbuminuria respectively. However, cholesterol and triacylglycerol increase significantly throughout the three stages of albuminuria. Hirano [57] concluded that the atherogenic lipoprotein profile becomes more prominent in type 2 diabetics when diabetic nephropathy advances. LDL-C

levels are increased with the progression of diabetic nephropathy. Our results showed that serum FGF21 levels were significantly associated with triacylglycerol levels and systolic BP. These results were consistent with a previous study[58] The serum level of FGF21 was significantly associated with systolic BP. Furthermore, subjects with high systolic BP exhibited a significantly higher level of FGF21.. High levels of triacylglycerol in the bloodstream, which exhibited an association with high FGF21 level, has been also linked to atherosclerosis, which, in turn, increases the risk of heart disease and stroke[59]. In fact, Lin and colleagues[60] have reported that increased serum FGF21 level is associated with coronary heart disease, which is independent of adverse lipid profile. Hence, FGF21 might serve as a biomarker for the risk of cardiovascular diseases.. In the present study, serum FGF21 was positively correlated with circulating triacylglycerol, and positively associated with body mass index. These findings are consistent with a previous study.[61] . Our results also showed a positive significant correlation between Fetuin-A and the lipid profile. This is in agreement with the study of Suchitra et al.[62] who stated that the higher serum fetuin-A levels were associated with metabolic syndrome and atherogenic lipid profile in patients with coronary artery disease. Blood glucose is not utilized by tissues resulting in hyperglycemia. Consequently fatty acids are mobilized from adipose tissue to meet the energy demands and in the process excess fatty acid accumulates in the liver (due to impaired uptake by the skeletal muscle) with the presence of adequate glycogen stores in the liver. This will promote triacylglycerol production, which stimulates the secretion of VLDL cholesterol. Further, long-term hyperglycemia causes generalized vascular endothelial damage, which reduces functional lipoprotein lipase (LPL) activity resulting in reduced catabolism of chylomicrons and VLDL and a decrease in HDL. Regarding the kidney function tests, there were a significant increase in serum urea and serum creatinine levels in the diabetic group with microalbuminuria, being more pronounced in macroalbuminuria group when compared to normoalbuminuria and control groups. This may be due to once microalbuminuria is present creatinine clearance declines at the rate that varies widely from patients to patients on an average; reduction may be 10-12 ml/min. Basement membrane thickening in diabetic nephropathy due to various factors affecting kidney function may be other cause for raised urea and creatinine[63]. These results in agreement with Jha et al.[63]. In addition, the levels of urinary albumin to creatinine ratio and microalbumin in the diabetic group with microalbuminuria were significantly elevated in diabetic nephropathy groups when compared to normoalbuminuria and control groups. This result in agreement with Hellemons et al.[64] who stated that diabetic nephropathy clinically involves the transition in albuminuria class (from normo- to microalbuminuria and from micro- to macroalbuminuria) or doubling of serum creatinine from baseline as indicators of nephropathy onset or progression. The pathophysiologic basis for elevated urinary albumin excretion entails the binding of glucose to proteins resulting in excessive protein glycosylation with the buildup of advanced glycosylated

end products. This leads to deposition of advanced glycosylated end products on the glomerulus resulting in renal and glomerular hypertrophy, mesangial matrix accumulation and thickening of glomerular basement membrane. This abnormality permits the leakage of low molecular weight proteins (albumin). This is the stage of microalbuminuria (incipient nephropathy) which could be reversible with good glycemic control. However, with persistent microalbuminuria, further leakage of protein in urine will result in overt diabetic nephropathy. Increased level of microalbuminuria is associated with increased risk of progressive kidney disease leading towards end-stage renal disease and cardiovascular morbidity and mortality in diabetic patients. Our result showed serum concentrations of FGF21 are elevated in patients with renal dysfunction, and correlates with renal function. The exact mechanism and physiological significance of increased serum FGF21 levels in patients with renal dysfunction are not clear, but there may be several possibilities. First, it may be possible that serum FGF21 concentrations are elevated due to limited clearance of FGF21 in urine in patients with decreased renal function. Second, elevated FGF21 played a causative role in renal injury, but currently, there is little evidence to support this hypothesis. Lastly, insulin resistance observed in renal failure lead to a compensatory increase in FGF21 levels. The relationship between serum FGF21 concentration and insulin resistance has been explored in patients with renal disease[65]. Serum fetuin-A level was significantly increased in type 2 diabetic patients with micro- and macroalbuminuria group relative to type 2 diabetic patients with normoalbuminuria supporting a possible association and involvement of fetuin-A in the pathogenesis of diabetes and diabetic nephropathy and the possibility of fetuin-A to be a good marker for diabetic nephropathy. Also, There was a positive significant correlation between fetuin-A and HOMA- IR indicating an important value of fetuin-A in expecting the degree of insulin resistance as a factor in the pathogenesis of T2DM and its complications. In this study the diabetic patients did not show a good glycemic control, therefore, they showed a positive correlation between serum FGF21 and fetuin-A levels and FPG and HbA1c levels, also, there was a positive correlation between serum FGF21 and fetuin-A levels with the urinary ACR, lipids, BMI and systolic pressure and HOMA-IR in the diabetic patients groups. The results suggest that FGF21 and fetuin-A could prove to be markers for the assessment of diabetic nephropathy in type 2 diabetic patients after standardization in a population-based study. The findings of this study have implications for the care of patients with T2DM. Patients and health care providers should give the highest priority to improving glycemic control sufficiently to maintain HbA1C values below 8.0 percent. If this can be achieved, the number of patients in whom microalbuminuria develops should decline substantially. The elevation in both serum FGF21 and Fetuin-A levels may simply reflect compensatory responses and reflect on the severity of the underlying renal inflammation and injury in type 2 diabetes, which would contribute to the development and progression of diabetic nephropathy.

CONCLUSION

Serum FGF21 and fetuin-A levels are strongly associated with insulin resistance in T2DM with nephropathy and could be promising useful biomarkers for predicting nephropathy progression, especially at early stages of diabetic nephropathy. Furthermore, they may serve as a tool to monitor the impact of prevention and intervention on renal damage.

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INFORMED CONSENT

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

CONFLICT OF INTEREST

Conflict of interest declared none.

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