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Fibroblast Growth Factor 21 as a possible metabolic marker of diabetic nephropathy in Type 2 diabetic patients

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ABSTRACT

Diabetic nephropathy in Type 2 diabetes is difficult to predict because the onset of diabetes is not usually well established. Accordingly, the aim of this study was to evaluate Fibroblast growth factor 21 (FGF21), a modulator of cellular activities, as a biomarker of progressive nephropathy in Type 2 diabetes. Eighty subjects were enrolled in this study: 20 normal controls were age and sex matched with 60 Type 2 diabetics. Diabetic groups were classified according to albumin /creatinine ratio (A/C ratio) into diabetic group with normoalbuminuria (A/C ratio<30mg/g), diabetics with microalbuminuria (A/C ratio=30-300mg/g) and diabetics with macroalbuminuria (A/C ratio >300 mg/g). In Type 2 diabetic groups with normo, micro and macroalbuminuria, there were significant increases (P<0.001) in the levels of FGF21 parallel to the significant elevations in the levels of the diabetic biomarkers (fasting plasma glucose, s.insulin, glycosylated hemoglobin, HOMA-IR), lipid profile parameters (total cholesterol, triacylglycerol, low density lipoprotein-cholesterol, very low density lipoprotein-cholesterol and atherogenic index 1&2) except high-density lipoprotein-cholesterol which showed a significant decrease (P<0.001) as compared to the control group. Serum levels of FGF21 as well as kidney function tests (s. cyctatin C, s. creatinin, s. urea, BUN) primarily cyctatin C were progressively increased (P<0.001) parallel to the degree of albuminuria as compared with the normal controls. In the diabetic macroalbuminuria group serum albumin levels showed a significant decrease (P<0.05). Furthermore, in diabetic micro and macroalbuminuria groups estimated glomerular filtration rate (eGFR) showed significant decreases (P<0.001) as compared to the control group. In all diabetic groups FGF21 was positively correlated with the diabetic biomarker, lipid profile parameters (except HDL-C) and kidney functions tests however; it was negatively correlated with HDL-C and eGFR. In addition, in diabetic micro and macroalbuminuria groups FGF21 showed significant positive correlations with microalbumin in urine and A/C ratio, while it showed significant negative correlations with serum albumin and urinary creatinine. These results concluded that FGF21 is associated with the progression of albuminuria in Type 2 diabetes mellitus and can be used as a metabolic biomarker for diabetic nephropathy in Type 2 diabetic patients.

Introduction

Diabetes mellitus Type 2 is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relatively insulin deficiency. Type 2 diabetes makes up about 90 % of cases of diabetes with the other 10% due primarily to diabetes mellitus Type1 and gestational diabetes. Approximately 40-50% of patients with Type 1 diabetes and 20-30% of patients with Type 2 diabetes develop diabetic nephropathy [1]. Diabetic nephropathy or diabetic kidney disease is a progressive kidney disease caused by damage to the capillaries of the kidney's glomeruli which leads to the hallmark feature of albumin in the urine [2]. Comparing the amount of albumin in the sample against its concentration of creatinine (A/C ratio) is used in diagnosis of the degree of albuminuria [3]. Microalbuminuria is defined as A/C

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ratio ≥ 3.5 mg/mmol (female) or ≥ 2.5 mg/mmol (male) or with both substances measured by mass, as an A/C ratio between 30 to 300 µg albumin/ mg creatinine. Nephropathy was defined as urinary A/C ratio ≥ 300 mg/g [4]. Microalbuminuria is often considered as a sensitive early marker for diabetic kidney disease (DKD) and is thought to precede the more detrimental events known in advanced stages of diabetic nephropathy [5].

Circulating biomarkers have been important in predicting development of diabetes and its complications as well as providing targets for therapy [6]. FGF 21 is detected in plasma, suggesting that it is secreted into circulation acting as a true hormone [7]. Unlike other members of the FGF family, FGF21 lacks a conventional heparin binding domain and thus can diffuse away from its tissue of origin and function as a hormone regulator in metabolic processes [8]. Furthermore, unlike the majority of the members of the FGF family, FGF21 does not have effects on cell proliferative and tumorigenic effects [9]. Fibroblast growth factor 21 acts as an endocrine factor that regulates glucose and lipid metabolism. It predicts cardiovascular events and mortality in Type 2 diabetic patients [10]. Higher expressions of FGF21 were found 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 [11]. The cofactor β-Klotho has a vital role for the FGF21 specificity of the target cells which increase the ability of FGFRs to bind FGF21 [12]. The FGF21–β-Klotho–FGFR complex acts by inducing MAP kinase phosphorylation in WAT [13]. The expression of FGF21 is controlled by different transcriptional factors such as peroxisome proliferator-activated receptor α (PPARα (PPARA) in the liver [12] and PPARγ (PPARG) in adipocytes [15].

Several authors suggested that circulating FGF21 levels are elevated in patients with obesity-related disorders, including Type 2 diabetes mellitus (DM) [16] and metabolic syndrome [17]. FGF21 improves insulin sensitivity, glucose and lipid homeostasis, also it has important role in preserving β-cell functions in diabetic animal models [18].

Accordingly, the present study aimed to assess the role of FGF21 in the pathogenesis of Type 2 diabetes with different degree of albuminuria. In addition, to examine the possibility of considering FGF21 as a predictor marker of diabetic nephropathy in Type 2 diabetic patients whom are at risk of that disease.

Subjects and Methods

Subjects

This study included eighty volunteers of both sexes (closely sex-matched by ratio in each subject group) classified as 20 normal healthy subjects and 60 Type 2 diabetic patients (The diabetic onset from 5 to 10 years) diagnosed according to Report of the Expert Committee on the Diagnosis and classification of Diabetes Mellitus 2006. They were selected from the outpatient's clinic of National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt. The participants in the diabetic groups were treated with oral hypoglycemic agents only. Hypoglycemic medications were withheld on the morning of the study. The patients were instructed not to engage in any vigorous exercise for at least 3 days before the study. None of the healthy subjects took any medications known to affect lipid or glucose metabolism. They had no known disease history and no abnormal laboratory findings.

Subjects included in this study were classified into the following:

Normal control: 20 normal healthy subjects

Diabetic normoalbuminuria: 20 Type 2 diabetic patients with A/C< 30 mg/g.

Diabetic microalbuminuria: 20 Type 2 diabetic patients with A/C 30 – 299 mg/g.

Diabetic macroalbuminuria: 20 Type 2 diabetic patients with A/C ≥ 300 mg/g.

Inclusion Criteria:

Type 2 diabetes, BMI < 30 kg/m².

Exclusion Criteria:

Patients with any history of smoking, alcohol habits, respiratory disorder or showed any clinical or laboratory signs of liver disease, thyroid dysfunction, chronic inflammation, and clinically significant inflammation and infectious diseases were excluded from the study.

Methods

Ten milliliters of venous blood samples were collected from patients and healthy controls in the morning after an overnight fasting as follows: Two milliliters of blood were added to tubes with potassium oxalate and sodium fluoride for assaying plasma glucose level. Two milliliters of blood were added to EDTA coated tubes for estimation of HbA1c and the rest of the blood sample was kept in clean glass tube without additive to clot at 37 °C for 20 minutes, and then centrifuged at 3000 rpm for 10 minutes. The samples were then separated into aliquots and stored at -80°C for estimation of insulin, lipids profile, cystatin C, creatinine, urea, albumin, and FGF 21.

Urine Specimens

Urine sample was taken from each subject in clean container. Five milliliters were centrifuged at 3000 rpm, the supernatants were separated in dry clean glass tubes and stored at -20ºC for the determination of microalbumin and creatinine levels.

Biochemical analysis

Diabetic biomarkers: Fasting plasma glucose (FPG) [19] was determined using Spinreact diagnostic kits. Glycated Haemoglobin (HbA1c) [20] was determined using Stanbio Laboratory, kits. Insulin [21] was determined by an enzyme-linked immunosorbent assay (ELISA) technique (DRG International insulin ELISA) according to the manufacturer's protocols. Insulin resistance was assessed using the homeostasis model assessment-insulin resistance index (HOMA-IR) by multiplying fasting plasma glucose (mmol/L) and fasting plasma insulin (µU/ml) divided by 22.5 [22].

Lipid profile parameters: Serum total cholesterol (TC) [23], high density lipoprotein cholesterol (HDL-C) [24] and triacylglycerol (TAG) [25] were determined by using a commercial assay Kits (Spinreact diagnostics, Egypt).

VLDL-C was calculated according to Friedewald *et al.* [26] formula:

$$\text{VLDL-c (mg/dl)} = \frac{\text{TAG conc.}}{5}$$

Serum low density lipoprotein was calculated as follows:

$$\text{LDL-C} = \text{TC- (TAG/5+HDL-C)}$$

Atherogenic index 1 = $\frac{\text{Total cholesterol}}{\text{HDL-C}}$

and Atherogenic index 2 = $\frac{\text{LDL-C}}{\text{HDL-C}}$

Kidney function tests: Human serum cystatin C [27] was quantitatively assayed using ELISA technique (R & D Systems Quantikine, USA) according to the manufacturer's protocols**.** Serum creatinine [28] and serum urea [29] were determined by using Diamond Diagnostics, Egypt kits. Blood urea nitrogen (BUN) in mg/dl was calculated from the equation $= \frac{\text{Urea mg/dl}}{2.14}$.

Estimated GFR (eGFR) was calculated using the MDRD equation and the MDRD-GFR Calculator program: (http://www.kidney.org/professionals/KDOQI/gfr_ calculator.cfm).

eGFR (ml/min/1.73m²) = 175x [serum creatinine (SI) x 0.011312] -1.154x [age}-0.203 x [1.212 if black] x [0.742 if female].

Serum albumin [30] was determined using Diamond diagnostics, Egypt kits. The microalbumin [31] was determined using AGAPPE diagnostics Switzerland GmbH kits. Urine creatinine [28] was determined using Diamond diagnostics, Egypt kits. Albumin/creatinine ratio (A/C ratio) in mg/g was calculated using the formula

$$= \frac{\text{microalbumin in urine (mg/L)}}{\text{creatinine in urine (mg/dl)}} \text{ X } 100.$$

Fibroblast growth factor 21(FGF21) was quantitatively assayed by ELISA technique according to the manufacturer's protocols. The kit was purchased from EIAab Wuhan EIAab Science Co. Ltd., (China) Catalog No: E0188h.

Statistical analysis:

Data are presented as mean±SD. The data were analyzed by one-way analysis of variance (ANOVA). A *P* value less than 0.05 was considered statistically significant. To compare the difference among the groups, post hoc testing was performed by the Bonferroni test. Pearson's correlation coefficient analysis was used to determine the correlations between fibroblast growth factor 21 and the different studied parameters. Statistical analysis was performed using Statistical Package for the Social Science (SPSS) for Windows (version 23, Chicago, IL, USA).

Table 1. General characteristics features and diabetic biomarkers (fasting plasma glucose, glycosylated hemoglobin, s. insulin, and HOMA-IR) in the different studied groups

Parameters \ Groups	Normal controls (n=20)	D. Normo-albuminuria (n=20)	D. Micro-albuminuria (n=20)	D. Macro-albuminuria (n=20)
Age (years)				
mean±S.D	55.00 ± 6.19	56.80 ± 6.70	57.50 ± 5.90	59.70 ± 6.00
% change		13.60	15.00	19.40
***P*<**		NS	NS	NS
Gender (Males/Females)	9/11	11/9	10/10	8/12
Systolic BP (mmHg)				
mean±S.D	123.10 ±6.79	125.75 ±7.30	137.14± 6.76	150.49±6. 44
% change		2.15	11.41	22.25
***P*<**		NS	0.01	0.001
Diastolic BP (mm Hg)				
mean±S.D	78.66 ± 5.09	80.49 ± 7.29	88.81±9.38	98.70 ± 5.16
% change		2.33	12.90	25.47
***P*<**		NS	0.05	0.001
FPG (mmol/L)				
mean±S.D	4.62±0.50	13.33±1.05	15.08±1.27	17.46±1.28
% change		188.53	226.41	277.92
***P*<**		0.001	0.001	0.001
HbA1c (%)				
mean±S.D	5.27 ±0.37	9.85±1.48	10.64±1.58	11.04±1.72
% change		86.91	101.89	109.49
***P*<**		0.001	0.001	0.001
Insulin (µU/ml)				
mean± S.D	6.14±0.37	11.48±1.80	12.04±1.85	19.08±2.40
% change		86.97	96.09	210.75
***P*<**		0.001	0.001	0.001
HOMA-IR				
mean ±SD	1.26±0.17	6.79±1.16	8.05±1.30	14.91±2.82
% change		438.89	538.89	1083.33
***P*<**		0.001	0.001	0.001

% change and *P*-values were calculated with respect to normal controls.

P<0.05: significant, *P*< 0.001: highly significant and NS: non-significant

Results

General characteristic and diabetic biomarkers

Data presented in **Table 1** showed that there were no significant changes concerning ages between the diabetic groups and the control group. However, in the diabetic groups with micro and macroalbuminuria systolic and diastolic blood pressure showed significant increases *(P*< 0.01, *P*< 0.001) compared to the control group (% changes for systolic:11.41, 22.25 and for diastolic: 12.9, 25.47 % respectively)

In diabetic patients with normo-, micro- and macroalbuminuria, FPG levels, HbA1c, insulin levels and HOMA-IR all showed significant increases (*P*< 0.001) with respect to normal controls. % changes for FPG were 188.53, 226.41 and 277.92 % respectively, % changes for HbA1c were 86.91, 101.89 and 109.49 % respectively, % changes for s. insulin were 86.97, 96.09 and 210.75 % respectively and % changes for HOMA-IR were 438.89, 538.89 and 1083.33 % respectively.

Fibroblast growth factor 21

Data presented in **Figure 1** demonstrated that there were significant increases in FGF 21 levels in diabetic patients with normoalbuminuria (*P*<0.05), in diabetic patients with micro- and macroalbuminuria (*P*<0.001) compared to normal controls (% changes: 12.86, 30.99 and 96.44 % respectively).

Lipid profile

Data presented in **Table 2** demonstrated that in diabetic patients with normo-, micro- and macroalbuminuria there were significant increases (*P*< 0.001) in TC, TAG, LDL-C, VLDL-C levels, atherogenic index 1 and 2 however, HDL-C significantly decreased (*P*<0.001) compared to normal controls. % changes for TC were 26.76, 32.94 and 37.58 % respectively, % changes for TAG were 122.84, 133.23 and 135.36 % respectively, % changes for HDL-C were -23.63, - 25.43 and - 26. 88 % respectively, % changes for LDL-C were 42.70, 53.37 and 62.49 % respectively, % changes for VLDL-C were 122.84, 133.23 and 135.36 % respectively, % changes for Atherogenic index 1 were 68.31, 78.17 and 86.97 % respectively and % changes for Atherogenic index 2 were 89.10, 104.49 and 119.23 % respectively.

Kidney function tests

Data presented in **Table 3** showed that in diabetic patients with normo-, micro- and those with macroalbuminuria had significantly higher (*P*< 0.001) levels of cystatin C (% changes: 17.61, 25.83 and 70.66 %, respectively) than the normal controls. Serum creatinine levels showed significant increases (*P*<0.05) in diabetic patients with normoalbuminuria; in diabetic patients with micro- and macroalbuminuria (*P*<0.001) with % changes 43.75, 68.75 and 159.38 % respectively, compared to normal controls. There were significant increases in s. urea levels in diabetic patients with normo- (*P*<0.05), micro- and macroalbuminuria (*P*<0.001) compared to normal control, with % changes 15.64, 50.88, 139.87 % respectively.

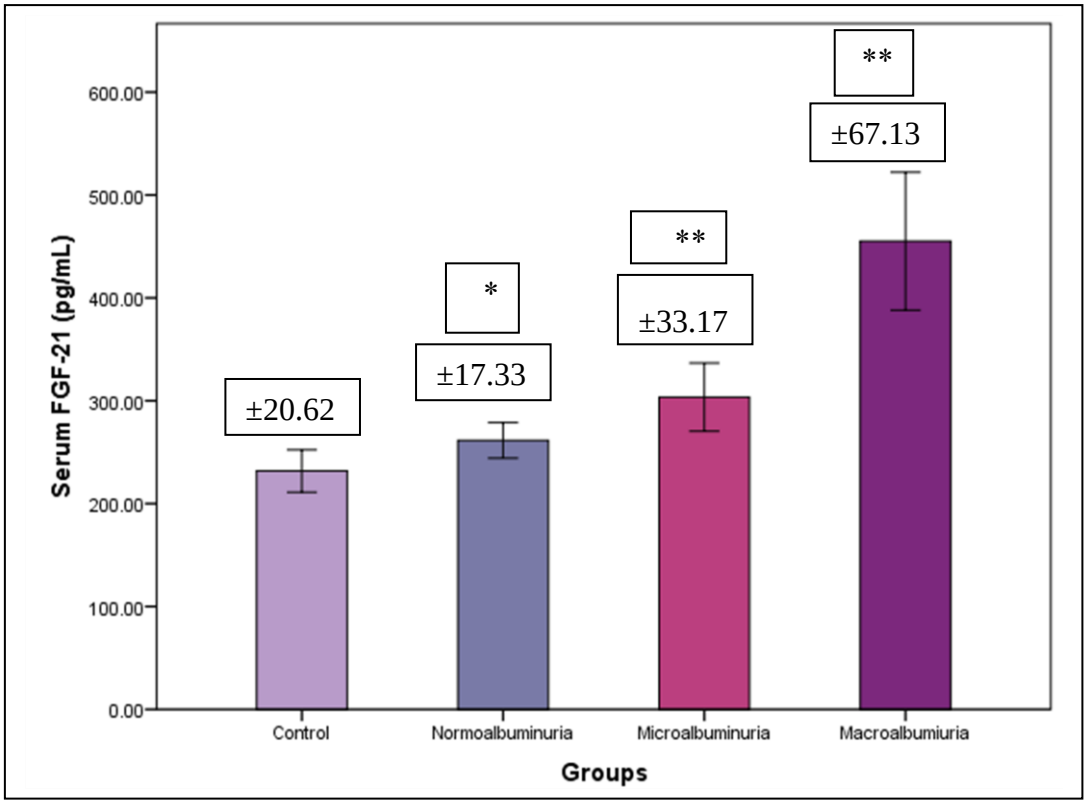

Fig 1: Serum fibroblast growth factor 21 level in normal control and diabetic groups with normoalbuminuria, microalbuminuria and macroalbuminuria. Values are given as mean ± S.D for groups of 20 individuals.

***** Statistically significant increase (*P* < 0.05).

** Statistically highly significant increase (*P* < 0.001).

Table 2. Lipid profile (total cholesterol, triacylglycerol, high density lipoprotein-cholesterol, low density lipoprotein - cholesterol, very low-density lipoprotein-cholesterol and atherogenic factors 1 and 2) in the different studied groups

Parameters \ Groups	Normal controls (n=20)	D. Normo-albuminuria (n=20)	D. Micro-albuminuria (n=20)	D. Macro-albuminuria (n=20)
TC (mg/dl)				
mean ±SD	163.50±17.27	207.25±31.37	217.35±47.73	224.95±45.79
% change		26.76	32.94	37.58
P<		0.001	0.001	0.001
TAG (mg/dl)				
mean ±SD	79.90±16.64	178.05±74.19	186.35±75.45	188.05±58.54
% change		122.84	133.23	135.36
P<		0.001	0.001	0.001
HDL-C (mg/dl)				
mean ±SD	58.60±7.07	44.75±9.19	43.70±8.33	42.85±5.49
% change		- 23.63	- 25.43	- 26.88
P<		0.001	0.001	0.001
LDL-C (mg/dl)				
mean ±SD	88.92 ±20.85	126.89±31.73	136.38±37.07	144.49±40.34
% change		42.70	53.37	62.49
P<		0.001	0.001	0.001
VLDL-C (mg/dl)				
mean ±SD	15.98 ±3.33	35.61±14.84	37.27±15.09	37.61±11.71
% change		122.84	133.23	135.36
P<		0.001	0.001	0.001
Atherogenic.Index1				
mean ±SD	2.84 ±0.50	4.78 ±1.06	5.06 ± 1.06	5.31 ±1.1 1
% change		68.31	78.17	86.97
P<		0.001	0.001	0.001
Atherogenic. Index 2				
mean ±SD	1.56 ±0.49	2.95 ±0.94	3.19 ±0.82	3.42 ± 0.99
% change		89.10	104.49	119.23
P<		0.001	0.001	0.001

% change and *P*-values were calculated with respect to normal controls.
P<0.05: significant, *P*< 0.001: highly significant and NS: non-significant

BUN levels showed significant increases in diabetic patients with normoalbuminuria (*P*<0.05), in diabetic patients with micro- and macroalbuminuria, (*P*<0.001) compared to normal controls with % changes, 15.65, 50.80, and 139.77 % respectively.

Levels of microalbumin in urine showed a non-significant increase in diabetic patients with normoalbuminuria, while it shows significant increases (*P*<0.001) in diabetic patients with micro- and macroalbuminuria compared to normal controls (% changes: 1157.31 and 4923.28 %, respectively). A non-significant decrease was recorded for creatinine in urine in diabetic patients with normoalbuminuria, while there were significant decreases (*P*<0.01) in diabetic patients with microalbuminuria % change -22.42 and with macroalbuminuria (*P*<0.001) % change -32.66 compared to normal controls.

Albumin/Creatinine ratios in urine showed a non-significant increase in diabetic patients with normoalbuminuria while, it showed significant increases (*P*<0.001) in diabetic patients with micro- and macroalbuminuria compared to normal controls, with % changes 1488.21 and 7111.29 %, respectively.

eGFR levels in diabetic patients with normoalbuminuria exhibited a non-significant decrease, while it showed significant decreases (*P*<0.001) in both diabetic patients with micro- and macroalbuminuria compared to normal controls, with % changes - 18.20 and - 48.71%, respectively. Serum albumin levels showed a non-significant change in diabetic patients with normo- and microalbuminuria, while it showed a significant decrease (*P*<0.05) in diabetic patients with macroalbuminuria, with % change -5.34%.

Correlation results

Data presented in **Table 4** showed that in the groups of diabetic patients with normoalbuminuria, significant positive correlations were recorded between serum FGF 21 and FPG (rs = 0.669, *P* < 0.001); HbA1c (rs = 0.588, *P* < 0.001); insulin (rs = 0.551, *P* < 0.001); HOMA-IR (rs = 0.623, *P* < 0.001); TC (rs = 0.453, *P* < 0.01); LDL-C (rs = 0.341, *P* < 0.05); VLDL-C (rs = 0.594, *P* < 0.001); TAG (rs = 0.594, *P* < 0.001); atherogenic index 1 (rs = 0.511, *P* < 0.001); atherogenic index 2 (rs = 0.421, *P* < 0.01); cystatin C (rs = 0.891, *P* < 0.001), creatinine (rs = 0.526, *P* < 0.001); urea (rs = 0.435, *P* < 0.01) and BUN (rs = 0.435, *P* < 0.01). However, significant negative correlations were recorded between FGF 21 and HDL-C (rs = -0.434, *P* < 0.01) and eGFR (rs = - 0.754, *P* < 0.001).

Table 3. Kidney function tests (s. cystatin C, s. creatinine, s. urea and blood urea nitrogen), Microalbumin, creatinine in urine, A/C ratio, e-GFR and serum albumin in the different studied groups

Parameters \ Groups	Normal controls (n=20)	D. Normo-albuminuria (n=20)	D. Micro-albuminuria (n=20)	D. Macro-albuminuria (n=20)
S. cystatin C (ng/ml)				
mean± S.D	590.50±59.78	694.50±107.29	743.00±88.62	1007.75±128.9
% change		17.61	25.83	70.66
P<		0.001	0.001	0.001
S. creatinine (mg/dl)				
mean± S.D	0.64±0.07	0.92±0.14	1.08±0.38	1.66±0.65
% change		43.75	68.75	159.38
P<		0.05	0.001	0.001
S. Urea (mg/dl)				
mean± S.D	22.70±2.58	26.25±2.73	34.25±6.00	54.45±6.83
% change		15.64	50.88	139.87
P<		0.05	0.001	0.001
BUN (mg/dl)				
mean± S.D	10.61±1.20	12.27±1.28	16.00±2.81	25.44±3.19
% change		15.65	50.80	139.77
P<		0.05	0.001	0.001
Microalbumin (mg/L)				
mean± S.D	16.75±3.77	17.40±2.98	210.60±27.67	841.40±88.85
% change		3.88	1157.31	4923.28
P<		NS	0.001	0.001
Creatinine in urine (mg/dl)				
Mean± S.D	125.55±33.51	102.60±27.82	97.40±26.23	84.55±13.85
% change		- 18.28	- 22.42	- 32.66
P<		NS	0.01	0.001
A/C Ratio (mg/g)				
mean± S.D	14.08±4.65	17.91±4.45	223.62±34.26	1015.35±160.36
% change		27.2	1488.21	7111.29
P<		NS	0.001	0.001
eGFR (mL/min/1.73m)				
mean± S.D	89.00±3.08	85.05±4.14	72.80±6.25	45.65±9.82
% change		- 4.44	- 18.20	- 48.71
P<		NS	0.001	0.001
S. albumin (g/dl)				
mean± S.D	4.12±0.14	4.14±0.20	4.06 ±0.26	3.90 ±0.54
% change		0.49	- 1.46	- 5.34
P<		NS	NS	0.05

% change and *P*-values were calculated with respect to normal controls.
P<0.05: significant, *P*< 0.001: highly significant and NS: non-significant

In the group of diabetic patients with microalbuminuria, significant positive correlations were recorded between serum FGF 21 and FPG (rs = 0.850, $P < 0.001$), HbA1c (rs = 0.777, $P < 0.001$), insulin (rs = 0.732, $P < 0.001$), HOMA-IR (rs = 0.819, $P < 0.001$); TC (rs = 0.350, $P < 0.05$); LDL-C (rs = 0.381, $P < 0.05$); VLDL-C (rs = 0.533, $P < 0.001$);TAG (rs =0.533, $P < 0.001$); atherogenic index 1 (rs = 0.652, $P < 0.001$), atherogenic index 2 (rs = 0.619, $P < 0.001$); cystatin C (rs = 0.821, $P < 0.001$); creatinine (rs = 0.753, $P < 0.001$); urea (rs = 0.864, $P < 0.001$); BUN (rs = 0.864, $P < 0.001$); microalbumin (rs = 0.764, $P < 0.001$) and A/C Ratio (rs = 0.763, $P < 0.001$). Moreover, significant negative correlations were found between FGF21 and HDL-C (rs = - 0.658, $P < 0.001$); creatinine in urine (rs = - 0.349, $P < 0.05$); eGFR (rs = - 0.916, $P < 0.001$); s. albumin (rs = - 0.402, $P < 0.01$).

In diabetic patients with macroalbuminuria group significant positive correlations were recorded between serum FGF 21 and FPG (rs = 0.936, $P < 0.001$); HbA1c (rs = 0.807, $P < 0.001$); insulin (rs = 0.944, $P < 0.001$); HOMA-IR (rs = 0.945, $P < 0.001$); TC (rs = 0.636, $P < 0.001$); LDL-C (rs = 0.634, $P < 0.001$); VLDL-C (rs = 0.669, $P < 0.001$); TAG (rs = 0.669, $P<0.001$); atherogenic index 1 (rs = 0.725, $P < 0.001$); atherogenic index 2 (rs = 0.691, $P < 0.001$); cystatin C (rs = 0.924, $P < 0.001$); creatinine ((rs = 0.870, $P < 0.001$); urea (rs = 0.940, $P < 0.001$); BUN (rs = 0.940, $P < 0.001$); microalbumin (rs = 0.900, $P < 0.001$) and A/C Ratio (rs = 0.913, $P < 0.001$). Moreover, significant negative correlations were found between FGF21 and HDL-C (rs = - 0.669, $P < 0.001$); creatinine in urine (rs = - 0.612, $P < 0.001$); eGFR (rs = - 0.947, $P < 0.001$) and s. albumin (rs = - 0.539, $P < 0.001$).

Table 4. Significant correlations between FGF21 and other biochemical parameters in the diabetic groups with different degree of albuminuria groups

Groups / FGF21	Normoalbumiuria		Microalbuminuria		Macroalbuminuria	
Parameters	**Rs**	**P**	**rs**	**P**	**Rs**	**P**
FPG (mmol/L)	0.669	<0.001	0.850	<0.001	0.936	<0.001
HbA1c (%)	0.588	<0.001	0.777	<0.001	0.807	<0.001
Insulin (µU/ml)	0.551	<0.001	0.732	<0.001	0.944	<0.001
HOMA-IR	0.623	<0.001	0.819	<0.001	0.945	<0.001
T. cholesterol (mg/dl)	0.453	<0.01	0.350	<0.05	0.636	<0.001
HDL-C (mg/dl)	-0.434	<0.01	-0.658	<0.001	-0.669	<0.001
LDL-C (mg/dl)	0.341	<0.05	0.381	<0.05	0.634	<0.001
VLDL-C (mg/dl)	0.594	<0.001	0.533	<0.001	0.669	<0.001
TAGs (mg/dl)	0.594	<0.001	0.533	<0.001	0.669	<0.001
Atherogenic Index 1	0.511	<0.001	0.652	<0.001	0.725	<0.001
Atherogenic Index 2	0.421	<0.01	0.619	<0.001	0.691	<0.001
S. cystatin C (ng/ml)	0.891	<0.001	0.821	<0.001	0.924	<0.001
S. creatinine (mg/dl)	0.526	<0.001	0.753	<0.001	0.870	<0.001
Urea (mg/dl)	0.435	< 0.01	0.864	<0.001	0.940	<0.001
BUN (mg/dl)	0.435	<0.01	0.864	<0.001	0.940	<0.001
Microalbumin (mg/L)			0.764	<0.001	0.900	<0.001
Creatinine in urine (mg/dl)			-0.349	<0.05	-0.612	<0.001
A/C Ratio (mg/g)			0.763	<0.001	0.913	<0.001
eGFR (ml/min/1.73m²)	-0.754	<0.001	-0.916	<0.001	-0.947	<0.001
S. albumin (g/dl)			-0.402	<0.01	-0.539	<0.001

Personal correlation (rs) was performed for FGF 21 levels and the indicated parameter. P<0.05: significant, P< 0.01: highly significant and P>0.05: non-significant.

Discussion

It is very important in managing diabetes mellitus to detect diabetic nephropathy as early as possible and to prevent its development. The role of FGF21 in the pathogenesis of Type 2 diabetic humans remains to be defined. Thus, the aim of this study was to evaluate the levels of FGF21 in the different stages of diabetic albuminuria in an attempt to examine the possibility of considering FGF21 as a metabolic marker of diabetic nephropathy in Type 2 diabetic patients whom are at risk of that disease.

As expected all of the diabetic patients enrolled in this study fulfilled the criteria of Type 2 namely, presence of hyperglycemia, hyper- insulinemia, insulin resistance and hyperlipidemia. Diabetic patients in this study were classified on the basis of the A/C ratios to diabetics with normoalbuminuria (<30mg/g), diabetics with microalbuminuria (30-299 mg/g), and diabetics with macroalbuminuria (≥300mg/g).

The pathogenic role of FGF21 on the diabetes-induced progression of nephropathy was reported previously [10-11]. These observations may suggest a. In the current study, as compared to normal control group, the progressive elevations in the FGF 21 levels in the diabetic groups of patients especially those with microalbuminuria (30.99%) and macro- albuminuria (96.44 %) may be attributed to observation that FGF21 is eliminated by the kidneys and its level increases as the stage of chronic kidney disease progresses as reported by Lin *et al.*, [32]. Recently [33] it was reported that the circulating FGF21 concentrations elevated with subjects with impaired glucose metabolism because of FGF21 resistance or the increased levels represent a compensatory response to facilitate glucose uptake that is blunted by insulin resistance. In all groups positive significant correlations were found between FGF21 and the diabetic biomarkers confirming the results of Lee *et al.*, [34].

Insulin resistance is important, since it is not only the most

powerful predictor of future development of type 2 diabetes, but it is also a therapeutic target once hyperglycemia is present [35]. In the present results, hyperglycemia established in Type 2 diabetic patients with normo, micro, and macro albuminuria, in spite of the presence hyper- insulinemia, signaling a defect in insulin action due to insulin resistance (elevated HOMA-IR values). This can be explained from the findings that increasing circulating FFAs metabolites such as diacylglycerols, fatty acyl CoA's or ceramides activates serine/threonine kinase, leading to the phosphorylation of serine/ threonine sites on insulin receptor substrates (IRS-1 and IRS-2), which then inhibits the insulin signaling cascade [36]. The progressive elevations in the levels of HbA1C in DM groups from normo- to macroalbuminuria when compared to normal control group represents integrated values for the glucose over the preceding 8 to 12 weeks and may associate with increased risk for development of microangiopathy in those patients. HbA1c has a special affinity for oxygen thereby causing tissue anoxia and plays a role in the causation of micro vascular complications [37].

Hyperlipidemia is an established risk factor in development of diabetic nephropathy in diabetics. Hyperlipidemia was confirmed in this study and it may be as a result of insulin resistance and defective action on lipoprotein metabolism. 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 in diabetic patients as demonstrated by Al-Jameil *et al.* [38]. In the present results the observed elevations in the serum levels of FGF 21 were parallel to the progression of hyperlipidemia in the diabetic groups compared with the normal control ones. This is in agreement with the study of Li *et al.* [39]. Previously, it was reported [40] that lipotoxicity and oxidative stress play a role of in the up-regulation of FGF21 synthesis. In addition, correlation results indicated that in all groups of diabetic patients there were positive significant correlations between FGF 21 and lipid profile parameters expect HDL-c which showed a negative significant correlation.

Cystatin C is a protein (120 amino acids) found in virtually all tissues and body fluids and removed from the blood stream by glomerular filtration in the kidney [41]. The present results indicated that in the diabetic group with normoalbuminuria compared to the normal control group, only the elevation in cyctatin C levels that signal an early renal impairment. The rest of the kidney function tests namely, s. creatinine, urea and BUN although they showed significant elevations, their mean levels were still within the acceptable normal values. In this regard cyctatin C appeared to be more sensitive marker than the rest of tested parameters predicting future defects in kidney functions in those diabetic patients as reported by Al-Saedy *et al.* [42]. This may be related to the finding that cystatin C concentrations is independent of age, sex, and muscle mass and its low molecular weight of 13 kDa and cationic nature enable its passage through the glomerulus [43]. Furthermore, it is not secreted but reabsorbed and catabolized by the proximal tubular cells without reentering the circulation [44].

Regarding the other two diabetic groups with micro and macroalbuminuria as compared to the control group there were progressive elevations in the levels of cyctatin C as well as s. creatinine, urea and BUN signaling gradual declines in renal functions parallel to the degree of albuminuria however, these elevations were more pronounced in the diabetic group of patients with macroalbuminuria. Our findings are also consistent with the previous results demonstrated by Brijesh and Saurav [45]. In the diabetic groups, circulating FGF21 levels were found to be progressively elevated and associated with the gradual declines in renal functions. This is may be because of the inability of the kidney to clear FGF21 in the urine [32]. Furthermore, FGF 21 showed significant positive correlations with all the parameters of kidney functions in the diabetic groups with different degree of albuminuria.

Microalbuminuria is the earliest marker, which is the predictor of incipient nephropathy in diabetic patients [46]. In the current study, an 1157.31% increase in urinary albumin was found in the diabetic microalbuminuria group while it showed a 4923.28% increase in the diabetic macroalbuminuria group as compared with the control group. These findings may be attributed to degradation of the glomerular basement membranes and hypertension in diabetic patients as demonstrated by Ghazalli [47]. Furthermore, it appears that 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]. Increasing levels of albumin in the urine have been established as an important determinant for renal complication of diabetic patients [49]. In the examined diabetic groups with micro and macroalbuminuria, there was an association between the elevations in FGF 21 and the progression in microalbuminuria, the decline in urinary creatinine and consequently the elevations in A/C ratios. Furthermore, FGF21 was positively correlated with microalbumin and A/C ratios while, negatively correlated with urinary creatinine in the diabetic groups with micro- and macro- albuminuria.

GFR is the best indicators of the degree of the renal damage in the early stages of diabetic nephropathy [50, 31]. In current study eGFR values were declined as the degree of albuminuria increased and this can be attributed to the finding that as glucose level increase, the eGFR decreased which leads to increasing the risk to Chronic Kidney Disease as stated by Belguith [51]. In addition, in the current study it was found that serum FGF21 levels increased progressively parallel to the declines in eGFR values suggesting that serum FGF21 elevation could be a reliable biomarker for eGFR decline. Supporting that FGF 21 levels was negatively correlated with eGFR.

Human serum albumin is the most abundant plasma protein synthesized mainly in the liver. It is a major soluble protein of the circulatory system, it has many

physiological, and pharmacyological functions [52]. In the present study, the declines in the levels of serum albumin were observed only in diabetic patients with macroalbuminuria, compared to normal controls. The present results were in agreement with that of Malawadi and Adiga [53]. Type 2 diabetes mellitus is a known state of insulin resistance affecting metabolism of carbohydrates, lipids as well as proteins and during insulin deficiency or resistance, fractional synthetic rate of albumin was decreased significantly and concomitantly fibrinogen synthesis was increased [54]. FGF 21 was correlated negatively with serum albumin and urinary creatinine.

Conclusion:

FGF21 plays a role in the pathogenesis of type 2 diabetes. In the diabetic groups of patients, the progressive increase in serum FGF21 levels was associated with poor glycemic control, insulin resistance as well as with the decline in kidney functions. Accordingly, FGF21 can be used as a useful metabolic marker of diabetic nephropathy in Type 2 diabetic patients whom at risk of that disease.

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