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ASSESSMENT OF CIRCULATING FIBROBLAST GROWTH FACTOR 23 IN TYPE 2 DIABETIC PATIENTS WITH NEPHROPATHY

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ABSTRACT

About one third of type 2 diabetics will eventually have progressive deterioration of renal function. Fibroblast growth factor 23(FGF23) is one of the main bone-derived endocrine hormones that plays many regulatory functions in different body systems especially in kidneys. In the current study, significantly increased FGF23 levels were observed in micro- and macroalbuminuric diabetic groups when compared to the control and normoalbuminuric diabetic groups. FGF23 found to correlate positively with duration of diabetes, serum creatinine and ACR, but correlate inversely with GFR in DN groups.Receiver operating characteristic curves revealed that for early detection of DN , the best cutoff values to discriminate DN and diabetic without nephropathy groups were 430.1 pg/mL for FGF23 with 80.8% sensitivity, 53% specificity, and area under the curve (AUC) = 0.773; 25 mg/g creatinine for ACR with 100% sensitivity, 95% specificity, and AUC = 1. ACR was more sensitive and specific than FGF23.therefore, we can conclude that FGF23 is associatedwith the different stages of DN but is less sensitive and specific than ACR for early detection of nephropathy in diabetic patients. In fact, additional studies are needed to assess the significance of changes in FGF23 levels during the course of DN.

KEYWORDS: *Fibroblast Growth Factor 23, type 2 diabetic nephropathy, albuminuria, Glomerular Filtration Rate.*

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INTRODUCTION

Diabetic nephropathy is characterized by an increased urinary albumin excretion in the absence of other renal diseases. The earliest clinical evidence of nephropathy is the presence of low but abnormal levels of albumin in the urine, which is known as microalbuminuria or incipient nephropathy[1]. FGF23 is primarily secreted by osteocytes and has several endocrine effects on mineral metabolism. It also inhibits secretion of parathyroid hormone (PTH) [2]. Intact FGF23 (approximately 32kDa) contains a proteolytic cleavage site (R176XXR179) recognized by a family of serine endopeptidase proprotein convertases. Cleavage at this locus generates N- and C-terminal fragments (approximately 18 and 14 kDa, respectively) that, with intact hormone, enter the circulation[3].Klotho is a 130-kDa transmembrane β-glucuronidase that catalyzes the hydrolysis of steroid β-glucuronides. Klotho is required for FGF23 to activate FGF receptors (FGFRs) and their downstream signaling molecules. FGF23 exerts its biological effects through activation of FGFRs in a Klotho-dependent manner[4].Fibroblast growth factor 23 (FGF23) has emerged as a new factor in mineral metabolism in chronic kidney disease (CKD)[5] .The kidney is an important target of FGF23, and the circulating levels of FGF23 have been found to increase in association with disease progression and cardiovascular events in chronic kidney disease and diabetic nephropathy[6] .This study aims to investigate the relationships between fibroblast growth factor 23 (FGF23) levels and albuminuria in type 2 diabetic patients and evaluate the diagnostic value of FGF23 as a biomarker for early detection of nephropathy in type 2 diabetic patients.

MATERIALS AND METHODS

This study included 160 subjects. Diabetic patients were recruited from the outpatient clinic of the National Institute for Diabetes and Endocrinology "NIDE", Cairo, Egypt. All subjects gave an informed written consent for participation and the study was approved by the ethics committee of the General Organization for Teaching Hospitals and Institutes. Diabetic patients were diagnosed according to criteria of WHO[7] which recommended that Glycated Hemoglobin (HbA1c) of 6.5% or higher or Fasting plasma glucose (FPG) level ≥126 mg/dl. Subjects were classified into four groups which are; group 1, healthy normal control subjects, which comprised40 (12 males, 28 females) healthy subjects matching the same age and socioeconomic status with diabetic subjects, group 2**,** T2DM with normoalbuminuria, this group included 42 (14males, 28 females)type 2 diabetic patients having Albumin/creatinine ratio (ACR) < 30 mg/g creatinine)**,**group3, T2DM with microalbuminuria, thi**s** group included 42 (8 males, 34 females) type 2 diabetic patients with ACR = 30 - 300 mg/g creatinine), and group 4, T2DM with macroalbuminuria,this group included 36 (20males, 16 females) type 2 diabetic patients with ACR > 300 mg/g creatinine. The last three diabetic groups were classified according to urinary albumin excretion. The first step in screening and diagnosis of DN is to measure albumin in a spot urine sample, which is collected either as the first urine in the morning or at random. All subjects underwent full history taking and clinical examination. For diabetic subjects, obese patients (BMI ≥ 30 Kg/ m2), Smokers & alcoholics were excluded.Patients with a previous history of chronic disease of the kidneys, pancreas, or liver, with other known existing diseases, active inflammatory disease, or who were receiving insulin were excluded as well. For control subjects, smokers, alcoholics, hypertensive, hyperlipidemic, obese, subjects suffering from any diseases or receiving any regular medications were excluded. For all subjects, Body mass index "BMI" was calculated as weight (Kg) / height (m^2)[8] .Waist circumference (WC) was taken with a tape measure horizontally at the umbilicus, midpoint between the lower rib margin and the iliac crest, while the subjects were in the standing position after normal expiration, and recorded to the nearest centimeter[9] .Blood pressure was measured three times, and the average value was considered for data analysis. Patients already on an antihypertensive medication or having average blood pressure ≥ 140/90 mmHg were considered hypertensive according to the study by Mancia[10].

Laboratory investigations

After 12 hours of overnight fasting, 10ml venous blood samples were collected from controls and diabetic patients into three types of vacutainer tubes and processed as follows: first vacutainer tube with ethylenediaminetetraaceticacid (EDTA) (lavender cap) without centrifugation (whole blood sample) for assaying glycosylated hemoglobin; second tube with potassium oxalate and sodium fluoride (gray cap) for assaying of plasma glucose; and third tube without additive (red cap) in which blood was centrifuged at 4000 rpm for 10 minutes Sera were rapidly separated and subdivided into two aliquots. One aliquot of sera was used to perform Kidney function tests; including urea, creatinine and Lipid profile; including TC, HDL-C, LDL-C and TG.The remaining aliquots of sera were stored at -80 °C for FGF23 determination. Hemolyzed samples were excluded.A first morning urine sample was collected from each subject into a sterile container and used for the determination of microalbumin and urinary ACR. FPG concentration was determined by the glucose method [11]using a kit obtained from Siemens healthcare diagnostics inc, HbA1c was determined using ion-exchange high-performance liquid chromatography (HPLC) fully automated system (Bio-Rad D-10 Hemoglobin testing system).Enzymatically, Lipid profile parameters were measured with commercial kits based on different techniques, atherogenic index was calculated from the ratio TC/HDL-C. Serum urea concentration was quantitatively measured according to Tiffany[12].Colorimetrically, the amount of Creatinine in serum &urine was quantitatively measured according to Vasiliades[13].GFR was estimated by using the modification of diet in renal disease (MDRD) abbreviated equation: [GFR = 186 × $(\text{serum creatinine})^{-1.154}$ × $(\text{age})^{-0.203}$ × (0.742 if female)]. The "MultiGEN" Microalbumin assay is used for determination of microalbumin according to**Burtis**[14]. The use of random urine (spot-check) samples in screening for micro- and macroalbuminuria may ensure better compliance than 24-hr urine collections. Recently, 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 ACR expressed in mg albumin/gm creatinine. ACR was measured by dividing the microalbumin concentration in mg/l over the urine creatinine concentration in g/l. ACR was thus expressed in mg/gm creatinine. Serum FGF23 was determined by assay that employs the quantitative sandwich enzyme immunoassay technique. The kit was provided byELISAkitassays®. Catalog no ELISAHu000060.

STATISTICAL ANALYSIS

Data were analyzed with **SPSS** version 21, the normality of data was first tested with one-sample Kolmogorov-Smirnov test. Graphs were drawn by graphpad prism version 7,Qualitative data were described using number and percent, association between categorical variables was tested using Chi-square test, Continuous variables were presented as mean ± SD(standard deviation) for parametric data and Median for non-parametric data. ANOVA test used to compare mean of more than 2 groups (parametric data) while Kruskal Wallis Test used for comparison of median of more than two groups (non-parametric data), Spearman correlation used to correlate continuous non parametric data.Simple linear correlation (Pearson's correlation) was also done, r value was considered weak if ($r < 0.25$), mild if ($0.25 < r < 0.5$), moderate if ($0.5 < r < 0.75$) and strong if ($r > 0.75$). Sensitivity and specificity at different cutoff point tested by ROC Curve.For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value).The results were considered non-significant when the probability of error is more than 5% ($p > 0.05$), significant when the probability of error is less than 5% ($p \leq 0.05$)& highly significant when the probability of error is less than 0.1% ($p \leq 0.001$).The smaller the p-value obtained, the more significant are the results.

RESULTS

Table 1 illustrates the descriptive statistics and ANOVA (P value) for anthropometric data. It shows that there was no significant difference between groups regarding the age, sex, BMI and WC.There was a statistically significant difference in the duration of diabetes among the micro- and macroalbuminuric diabetic groups as compared to normoalbuminuric diabetic group. Moreover, the macroalbuminuric diabetic group showed a significant difference in the duration of diabetes as compared to microalbuminuric diabetic group.A significant higher systolic blood pressure (SBP) levels were observed in micro-, and macroalbuminuric diabetic groups as compared to the control group and normoalbuminuric diabetic group.Diastolic blood pressure (DBP) levels were significantly higher in macroalbuminuric diabetic group compared to each the other groups.

Table 1
Comparison between the different studied groups regarding anthropometric data.

Parameters		Group (1)n=40	Group (2) n=42	Group (3) n=42	Group (4) n=36	p-value
Age (year)	M ±SD	50.22±4.46	50.76±5.65	50.59±4.35	50.38±3.46	p=0.956
	Range	41.00-59.00	39.00-62.00	44.00-60.00	43.00-58.00	
Sex Male	n (%)	12 (30.0%)	14 (33.3%)	8 (19.0 %)	20 (55.6 %)	p= 0.052
Female		28 (70.0%)	28 (66.7 %)	34 (81.0 %)	16 (44.4%)	
BMI (kg/m2)	M ±SD	26.19± 1.96	27.49± 1.96	26.84± 2.06	24.96± 2.4	p=0.062
	Range	20.10-29.00	20.06-29.41	21.51-29.76	20.99-29.69	
WC (cm)	M ±SD	93.2±5.76	94.26±4.32	96.11±7.30	95.88±5.91	p=0.092
	Range	80-100	85-103	70-107	80-109	
SBP (mmHg)	M ±SD	119±7.77	122.33±13.38	129.04±15.74 ab	135.27±15.39 abc	p=≤.001**
	Range	100-130	100-150	110-170	100-170	
DPB (mmHg)	M ±SD	77±6.58	80.71±9.47	81.42±8.20	87.77±9.66abc	p=≤.001**
	Range	65.00-90.00	60.00-100.00	70.00-100.00	70.00-100.00	
Duration Of DM	M ±SD	-	5.23±1.10	6.21±1.17b	7.22±1.43bc	p=≤.001**
	Range	-	3.00-7.00	4.00-9.00	5.00-10.00	

n (number of subjects) , * Significant p ≤0.05 **highly significant p≤0.001. Values are represented as M ± SD (Mean ± standard Deviation) or percentage (%), group 1 (control), group 2 (diabetic patients with normoalbuminuria), group 3 (diabetic patients with microalbuminuria), and group 4 (diabetic patients with macroalbuminuria).a significantly different from control group, b significantly different from group 2, c significantly different from group 3.

Abbreviation

DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure Table 2 shows the descriptive statistics and comparison between all the studied groups considering fasting glucose level and HbA1c.The mean levels of FPG and HbA1c% were significantly higher in normo-, micro-, and macroalbuminuric diabetic groups as compared to the control group.

Table 2
Descriptive statistics and comparison between all groups regarding the glucose monitoring profile

Parameters		Group (1) n=40	Group (2) n=42	Group (3) n=42	Group (4) n=36	p-value
FPG (mg/dL)	M ±SD	96.97±8.61	182.30±43.27 [a]	220.97±65.29 [ab]	274.38±92.67 [abc]	p=≤.001**
	Range	79-109	110-319	145-414	139-572	
HbA1c (%)	M ±SD	5.36±.34	8.55±1.82[a]	9.67±1.92[ab]	11.31±1.59[abc]	p=≤.001**
	Range	4.60-5.90	6.50-13.20	6.10-14.40	8.50-14.80	

Values are represented as M ± SD (Mean ± standard Deviation); group 1 (control), group 2 (diabetic patients with normoalbuminuria), group 3 (diabetic patients with microalbuminuria), and group 4 (diabetic patients with macroalbuminuria).a significantly different from control group, b significantly different from group 2, c significantly different from group 3.* Significant p ≤0.05 **highly significant p≤0.001.

Abbreviation
FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin.

Table 3
Descriptive statistics and comparison between all groups regarding the lipids profile

Items		Group (1) n=40	Group (2) n=42	Group (3) n=42	Group (4) n=36	p-value
TC (mg/dL)	M ±SD	190.95±27.7	223.71±23.9[a]	240.88±38.7[a]	271.22±99[abc]	p≤.001**
	Range	135-273	181-286	148-310	182-737	
TG (mg/dL)	Median	121	163.50 [a]	153.50 [a]	159 [a]	p≤.001**
	Range	53 - 199	101 -265	61-490	77 -557	
HDL-C (mg/dL)	M ±SD	44.65±7.01	42.64±6.95	43.50±7.66	38.58±9[abc]	p=0.005*
	Range	33.00-61.00	28.00-58.00	32.00-63.00	15.00-64.00	
LDL-C (mg/dL)	M ±SD	121.97±27.8	146.16±22 [a]	158.90±43.2[ab]	190.55±82.1[abc]	p≤.001**
	Range	73-199	101-203	58-226	65-562	
Atherogenic Index	M ±SD	4.37±.86	5.36±0.91[a]	5.73±1.44[a]	7.33±3.06[abc]	p≤.001**
	Range	2.70-6.40	3.80-8.00	2.60-8.70	3.80-21.6	

Values are represented as M ± SD (Mean ± standard Deviation) or median; group 1 (control), group 2 (diabetic patients with normoalbuminuria), group 3 (diabetic patients with microalbuminuria), and group 4 (diabetic patients with macroalbuminuria).a significantly different from control group, b significantly different from group 2, c significantly different from group 3

Abbreviations
TC, total cholesterol; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 4
Comparison between the different studied groups regarding serum urea, creatinine, urine microalbumin, eGFR and urinary ACR.

Parameters	Group (1) n=40	Group (2) n=42	Group (3) n=42		Group (4) n=36	p-value
Urea (mg/dL)	M ±SD	19.90±3.69	26.33±6.41[a]	29.81±9.45 [ab]	33.25±9.44[abc]	p≤.001**
	Range	13.00-27.00	15.00-45.00	12.00-58.00	19.00-57.00	
serum Creatinine (mg/dL)	M ±SD	0.90±0.19	1.06±0.19 [a]	1.18±0.28[ab]	1.43±0.20[abc]	p≤.001**
	Range	0.50-1.30	0.70-1.50	0.80-1.80	1.00-1.90	
Urine microalbumin (mg/dL)	Median	10.45	19.30 [a]	128 [ab]	552.50 [abc]	p≤.001**
	Range	0.55 -37.80	4.90 -44.80	12 -1513	205 -1917	
eGFR (mL/min/1.73 m2)	M ±SD	83.06±27.61	67.15±17.74 [a]	57.51±12.70 [ab]	48.94±11.9[abc]	p≤.001**
	Range	55.2-154.2	50.3-129.6	38-80.1	29.90-82.80	
ACR (mg/g Cr.)	Median	7.50	12.10 [a]	106.50 [ab]	562 [abc]	p≤.001**
	Range	0.80-20.20	3.52 -27.00	33.40 -274	313 -1260	

shows a comparison between the different studied groups regarding serum urea, creatinine, urine microalbumin, eGFR and urinary ACR. Values are represented as M ± SD (Mean ± standard Deviation) or median; group 1 (control), group 2 (diabetic patients with normoalbuminuria), group 3 (diabetic patients withmicroalbuminuria), and group 4 (diabetic patients with macroalbuminuria).a Significantly different from control group, b Significantly different from group 2,c Significantly different from group 3.* Significant p ≤0.05 **highly significant p≤0.001.

Abbreviations
eGFR, estimated glomerular filtration rate, ACR, albumin creatinine ratio. There was a significant increase in FGF23 levels in micro- and macroalbuminuric diabetic groups compared to controls and normoalbuminuric groups. Significant high levels were shown in normoalbuminiric diabetic compared to control healthy group. Macroalbuminuric diabetic patients showed a pronounced increase in the levels of FGF23 when compared to the microalbuminuric diabetic group.

Table 5
Comparison between the different studied groups regarding serum FGF 23

Parameter		Group (1) n=40	Group (2) n=42	Group (3) n=42	Group (4) n=36	p-value
FGF23 (pg/mL)	Median	374.60	428.05 [a]	479 [ab]	539.90 [abc]	p≤.001**
	Range	226.8- 446.5	205 -567.3	319 -654	343.5- 1356	

Values are represented as M ± SD (Mean ± standard Deviation) or median; group 1 (control), group 2 (diabetic patients with normoalbuminuria), group 3 (diabetic patients with microalbuminuria), and group 4 (diabetic patients with macroalbuminuria).a significantly different from control group, b significantly different from group 2, c significantly different from group 3

Abbreviations

FGF23, fibroblast growth factor23. Table 6 illustrates the correlations between FGF23 and other parameters in patients with nephropathy. Pearson correlation analysis showed that FGF23 concentrations significantly positively correlated with the duration of diabetes (r = 0.313, P =0.005), serum Creatinine(r =0.269, P = 0.017) and urinary ACR (r =0.251, P = 0.026).FGF23 levels negatively correlated with eGFR (r = -0.277, P = 0.014).

Table 6
Correlations between FGF23 (pg/mL) and other parameters in patients with nephropathy

Items	FGF23 (pg/mL) (n=78)	
	r	p
Age (years)	0.068	0.553
BMI (kg/m2)	0.134	0.241
WC (cm)	-0.051	0.655
SBP (mmHg)	-0.037	0.746
DPB(mmHg)	0.176	0.123
Duration Of DM	0.313	0.005*
FG (mg/dL)	0.049	0.668
HbA1c (%)	0.098	0.394
TC (mg/dL)	0.023	0.843
TG (mg/dL)	0.040	0.726
HDL-C (mg/dL)	-0.146	0.202
LDL-C (mg/dL)	0.030	0.794
Atherogenic Index	0.154	0.179
Urea (mg/dL)	0.205	0.071
serum Creatinin	0.269	0.017*
Urine microalbumin	0.202	0.077
ACR (mg/g Cr.)	0.251	0.026*
eGFR (mL/min/1.73 m2)	-0.277	0.014*

r, Pearson correlation coefficient. *P-value: correlation is significant at the 0.05 level.

ROC curves were carried out to assess the diagnostic performance of FGF23, whether they are more sensitive and specific than ACR, and which of them is more sensitive or more specific in the diagnosis of nephropathy. Table 7 represents a comparison between the output data of ROC curves for FGF23 and ACR. A cutoff value of FGF23 >430.1 pg/mL was determined for discriminating between the diabetic patients with and without nephropathy. A cutoff value of ACR >25 mg/g creatinine was determined for discriminating between the diabetic patients with and without nephropathy.A serum level of FGF23 > 430pg/mL predicted the presence of nephropathy with 80.8 % sensitivity, 53% specificity, and AUC = 0.773 with 70.8% accuracy. And urinary ACR of >25mg/g creatinine predicted the presence of nephropathy with 100% sensitivity, 53% specificity, and AUC = 1 with 98.8% accuracy. These results illustrate that the urinary ACR is more specific than FGF23 in the early prediction of DN.

Table 7
FGF23 and ACR for prediction of patients with nephropathy.

Item	AUC	95% Confidence Interval		p-value	Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
		Lower Bound	Upper Bound							
FGF23 (pg/mL)	0.773	0.691	0.856	≤.001	430.1	80.8%	53%	75.9%	59.5%	70.8%
ACR (mg/g Cr.)	1.00	1.00	1.00	≤.001	25	100%	95%	97.5%	100%	98.3%

PPV, positive predictive value; NPV, negative predictive value; accuracy = number of correctassessments/number of all assessments; AUC, area under the curve

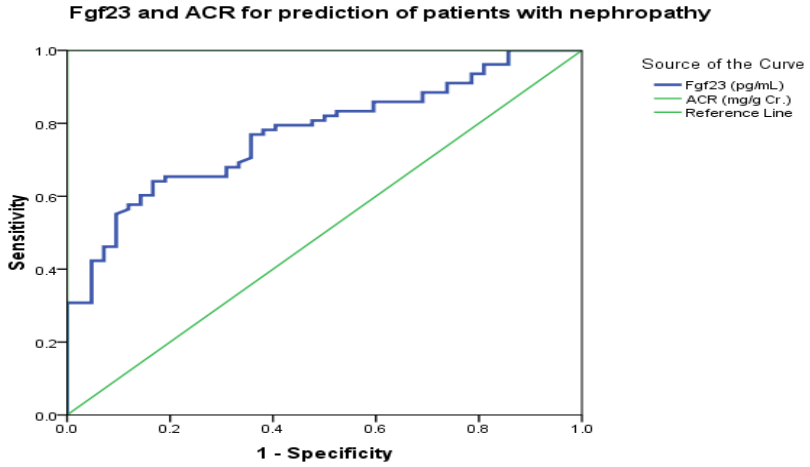

Figure1
ROC curve analysis of FGF23 and ACR in diabetic patients withnephropathy versus diabetic patients withoutnephropathy group.

DISCUSSION

Kidney disease is a critical determinant of outcomes in diabetes, and diabetic nephropathy remains a key area of focus for both preclinical and clinical research. Diabetic nephropathy affects 30–40% of people with diabetes, and is the leading cause of end-stage kidney disease The mechanisms by which hyperglycaemia promotes the development of nephropathy are now relatively well understood; however, the hope that an increased understanding of the pathogenesis of diabetic renal disease would lead to effective targeted strategies has largely yet to be realized [15]. Moderately increased albuminuria is widely accepted as the first clinical sign of diabetic nephropathy. However, previous morphometric studies and the autopsy study by Klessens[16]have clearly demonstrated that by the time moderately increased albuminuria is evident, the kidneys in some diabetic patients have already undergone glomerular and tubulointerstitial damage, which indicates that it is not a sensitive marker for diabetic nephropathy. Hence, there is a need for new biomarkers of early diabetic nephropathy [17]. The emergence of fibroblast growth factor 23 as a potentially modifiable risk factor in CKD has led to growing interest in its measurement as a tool to assess patient risk and target therapy [3]. A report by Fliser[18] suggests that FGF-23 is related to CKD in a nondiabetic CKD population. However, it is not clear whether the same relationship is observed in the setting of diabetic nephropathy (DN) [19]. In this study, we wished to assess significance of changes in FGF23 levels during the course of DN. No significant difference was observed between the ages, sex, BMI and WC of the studied groups. The duration of diabetes in the microalbuminuric diabetic group was significantly increased compared to the normoalbuminuric diabetic group, and this difference was even more pronounced in the macroalbuminuric group. In the current study, the levels of FG and HbA1c in the microalbuminuric diabetic group were significantly increased compared to diabetic normoalbuminuric and control groups this difference was more significantly in the macroalbuminuric group, These findings are in agreement with the previous studies, which have suggested that hyperglycemia is the driving force for the development ofDN [20]. Elevated HbA1c was associated with the development of microangiopathy in diabetes. Perhaps this is because HbA1c has a special affinity for oxygen, causing tissue anoxia and contributing to microangiopathy[21]. Poor glycemic control may have a significant role in the progression of DN [22]. In the current study, lipid patterns showed that the median serum levels of TG were significantly higher in macro-, micro- and normoalbuminuric diabetic groups than in the control. The mean serum level of LDL-C was significantly higher in macroalbuminuric diabetic groups than in the control and in the normo- and microalbuminuric groups. These results met the findings of Tseng[23] who concluded that TG increases significantly throughout the three stages of albuminuria and also match results of Inci[24] that LDL-C levels were found to be significantly different among the groups in their study about diabetic nephropathy with different stages of albuminuria. On the other hand, the mean serum level of HDL-C was significantly lower in the macroalbuminuric diabetic groups than in the control and in the normo- and microalbuminuric groups. TC/HDL-C ratio was significantly higher in the macroalbuminuric diabetic group than that observed in micro- and normoalbuminuric diabetic groups, each of them was significantly higher than in normal control subjects. These findings were in agreement with Dwivedi [25] who studied the effect of oxidative stress on DN progression and found that the level of each of TC, TG and LDL in DN group was significantly increased than the control group. Also, they found a significant decrease in HDL level in DN group. Our results also agree with Jacobs[26] who concluded that the prevalence of dyslipidemia was high in type 2 diabetic patients in comparison to healthy subjects. Suchitra[27]supported our results, they concluded that T2DM and diabetic nephropathy are associated with dyslipidemia which was more pronounced in diabetic nephropathy. Dabla[28]stated in his article about renal function in diabetic nephropathy that serum markers of glomerular

filtration rate and microalbuminuria identify renal impairment in different segments of the diabetic population. This agrees with our study, in which there was a reduction in GFR levels in the macroalbuminuric and microalbuminuric diabetic groups compared to the control and normoalbuminuric diabetic groups, and ACR was increased in the microalbuminuric and macroalbuminuric diabetic groups compared to control and normoalbuminuric diabetic groups. These results came also in accordance with those reported by Abid[29] stating that, in type 2 diabetic patients with microalbuminuria, the elevated levels of urinary albumin and ACR, together with the reduced levels of GFR, fulfill the characteristics of microalbuminuria. In our current study, median serum FGF23 levels significantly increased in macro-, microalbuminuric diabetic patient than normoalbuminuric diabetic group and control group. In agreement of these results, Fliser[18] data suggested that serum FGF-23 is related to the risk of CKD progression in macroalbuminuric DN. This relationship remained even after adjustments for the main confounding variables, such as sex, race, renal function, proteinuria, and intact PTH level. Inci[24]found in there study that, the serum FGF23 level was significantly different between patients with DN and healthy controls, and this met our results in this research. In our study, there was a negative correlation between FGF23 and GFR(r =-0.277; P=0.014). On the other hand, FGF23 positively correlated with duration of DM (r =0.251; P=0.026) and serum Our study met the studies of (Shigematsu[30,] Gutierrez[31,] Larsson[32]) who found that FGF23levels increase progressively as GFR declines beginning in early CKD. These results also came in accordance with Nitta[4] who stated that Plasma FGF23 levels have been found to be negatively correlated with eGFR. In accordance with Titan[5] who found that, Serum FGF-23 showed a significant association with serum creatinine, our study also proved that there was significant positive correlation between FGF-23 and serum creatinine. In correlation analysis of Inci[24] results, a significant positive correlation was found between FGF23 and UACR levels. This result met ours which also showed a significant positive correlation was found between FGF23 FGF23 may directly contribute to glomerular damage and enhanced proteinuria because the kidney is the primary target organ for FGF23. FGF23 signaling, however, is localized to the tubuli, where its co-receptor Klotho is expressed, so this scenario would require Klotho-independent FGF23 signaling. This requirement was evidenced in a study by Faul[33] Further studies should examine whether FGF23 modulates glomerular basement membrane characteristics. Alternatively, FGF23 may reflect vascular status that secondarily aggravates a decline in renal function or proteinuria [34]. Whether FGF-23 actually contributes to CKD progression or is only a marker of its risk remains an important and unresolved question. Most available data on FGF-23 and the risk of mortality and cardiovascular and renal morbidity rely on epidemiologic studies. More substantial proof of a causal effect between FGF-23 and these conditions is still lacking as stated by Titan [5]. Suggesting that CKD might be a state of high FGF-23 and low klotho levels,despite the low levels of klotho, FGF-23 might bind to FGF receptors with sufficiently high affinity in conditions of very high concentration [35]. On the other hand, some authors believe that FGF-23 might influence CKD progression by aggravating other mineral metabolism parameters that also modulate CKD progression. Animal and clinical studies suggest that FGF23 excess causes a decrease in $1,25(OH)_2$ vitamin D by inhibiting α -hydroxylase activity, favoring the occurrence of severe hyperparathyroidism [36].Vitamin D deficiency is possibly involved in the progression of CKD [37].

CONCLUSION

We can conclude that FGF23 is associated with the different stages of DN but is less sensitive and specific than ACR for early detection of nephropathy in diabetic patients

Recommendations

In fact, additional studies are needed to assess the significance of changes in FGF23 levels during the course of DN. Further in vivo studies on animal and cell culture models should be conducted to investigate the accurate mechanisms of FGF23 in the pathogenesis of DN. In addition,more researches should examine whether treatments that reduce FGF-23 serum levels or attenuate its pathogenic action provides survival benefits in patients with CKD.

CONFLICT OF INTEREST

Conflict of interest declared none.

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