The Egyptian Journal of Hospital Medicine (July 2024) Vol. 96, Page 2615-2622

Serum Fibroblast Growth Factor 21 and Its Relation to Diabetic Kidney (DKD) Disease and Cardiovascular Risk (ASCVD) in Type 2 Diabetic Patients

Khalid Elbana, Usama[1] Ahmed Khalil[1], Abdalla Abdel Aziz Abdalla[1], Mohammed Samy Fawzy[2], Ayman Magd-Eldin Mohamed Sadek[1], Mohammad Helal Ragheb Helal[1], Noha Usama Khalil[1]

[1]Department of Internal Medicine, [2]Department of Biochemistry, Faculty of Medicine, Zagazig University

Corresponding author: Khaled Ahmed El-Banna,

Email: khaledelbanna76@gmail.com, **Mobile:** +201118262030

ABSTRACT

Background: In both industrialized and developing nations, diabetic nephropathy is a major contributor to renal failure and the primary cause of death for those with diabetes. Fibroblast Growth Factor 21 **(**FGF21) mainly regulates metabolism to improve cellular glucose absorption, insulin sensitivity, and lipid metabolism. It also plays a crucial role in maintaining β-cell activities. Moreover, in individuals with type 2 diabetes, FGF21 levels rise in direct proportion to the advancement of the albuminuria stage.

Aim: This study aimed to investigate relation of serum level of FGF21 to different degrees of albuminuria in T2DM and to detect its impact on cardiovascular risk in those patients.

Patients and methods: This case-control study included 120 volunteer subjects who were divided into: Group A that included 30 healthy control subjects and group B that included 90 T2DM patients. Group B was further classified according to urinary albumin excretion into: Group B1: with normoalbuminuria, group B2: microalbuminuria and group B3: with macroalbuminuria. FGF21 was measured by ELISA and ASCVD risk score was calculated for all subjects.

Results: Highly statistically significant increase in serum FGF21 levels and ASCVD risk were found in group B1, B2 & B3 compared to group A, in group B3 compared to group B1 & B2 and in group B2 compared to group B1. Serum level of FGF21 is positively correlated with the ASCVD risk score in type 2 diabetic patients.

Conclusion; Elevated FGF21 and prolonged duration of diabetes are the main independent predictors of albuminuria in T2D patients. Increased FGF21 was associated with the ASCVD risk in type 2 diabetic patients.

Keywords: FGF21, T2DM, DKD, ASCVD, Albuminuria.

INTRODUCTION

Diabetes mellitus (DM) is defined as a chronic, progressive illness with systemic hyperglycemia. It is a major health issue that is becoming more commonplace globally. Recent diabetes data show that five hundred and seventy-nine million people (20–79 years old) have diabetes. This figure is projected to rise to 643 million by 2030 and 783 million by 2045 **(1).**

Many people believe that microalbuminuria is a sensitive early sign of kidney disease caused by diabetes (DKD) and that it occurs before the more harmful symptoms that are identified in the later stages of diabetic nephropathy **(2)**. Atherosclerotic cardiovascular disease development is associated with albuminuria. It is noteworthy that people with or without diabetes who have albuminuria have higher rates of cardiovascular morbidity and death **(3)**.

There are 22 members of the fibroblast growth factors family, and it has been shown that the majority function as paracrine factors. Many biological functions, including as wound healing, angiogenesis, cell differentiation, and proliferation, are facilitated by fibroblast growth factors (FGFs) **(4)**.

The circulating protein known as 181 amino acids make up human fibroblast growth factor 21 (FGF21) acids (w20 k Da) and is generated from a mature protein with 209 amino acids that the FGF21 gene, which is located on chromosome 19, encodes. The discovery of FGF21 occurred in 2000 **(5)**.

FGF21, which is produced by hepatocytes and excreted by the kidney, is mostly used as a metabolic regulator to improve the uptake of glucose by cells, insulin sensitivity, and lipid metabolism. It also plays a crucial role in maintaining β-cell activities. On the other hand, FGF21 synthesis may be aided by stressors, both internal and external, including oxidative stress on mitochondria or hypoxia that are connected to the pathogenesis of DKD. Furthermore, in people with type 2 diabetes, the levels of FGF21 rise in direct proportion to the advancement of the albuminuria stage **(6)**.

Serum FGF21 levels is strongly correlated with early-stage diabetic kidney impairment in the high-risk fraction of T2D patients. The relationship between FGF21 levels in serum and subclinical stages of diabetic nephropathy could provide insights into the early identification and mitigation of advanced chronic diabetes microvascular consequences by efficacious FGF21-targeted treatment **(7).** While, DKD continues to be the most common etiology of end-stage renal disease, which finally requires kidney replacement therapy for an extended period of time, has not decreased in occurrence over the previous 30 years. Numerous studies have been conducted to enhance the prediction of DKD start and development with is the goal of reducing disease prevalence. While, as the most commonly used indicators

Received:24/04/2024
Accepted:24/06/2024

of diabetic kidney disease (DKD), albuminuria and estimated glomerular filtration rate (eGFR) have drawbacks that have led researchers to look for other biomarkers that could enhance risk classification [8].

Several studies suggested that patients with diseases linked to fat, such as metabolic syndrome and type 2 diabetes, have higher levels of circulating FGF21, which may indicate a relative FGF21 resistance. But, FGF21 benefits for the cardiovascular system, especially for lipoprotein metabolism and atherogenesis, and its impact on cardiovascular risk is not well studied [9]. In contrast, few studies showed no significant relation between FGF21 and glycemic control [10] and diabetic nephropathy [11].

This study aimed to examine the association between the blood level of FGF21 and different levels of albuminuria in type 2 diabetes and to examine the possibility of considering FGF21 as an early marker for diabetic kidney disease and to detect its impact on cardiovascular risk in those patients.

PATIENTS AND METHODS

Patients: This case-control study was carried out at Internal Medicine and Clinical Biochemistry Departments, Faculty of Medicine, Zagazig University on 120 volunteer subjects divided into Group A (Control group) that included 30 healthy subjects and group B (Diabetic group) that included 90 type 2 diabetes mellitus (T2DM) individuals who met the ADA criteria for diabetes diagnosis [12]. They were matched with the control group as regards age, sex, waist circumference and BMI. This group was further classified according to urinary albumin excretion based on KDIGO clinical practice guidelines [13] into group B1 that comprised 30 patients with T2D having non-albuminuria, or normal to slightly elevated albuminuria (UACR <30 mg/g), group B2 that contained thirty T2D individuals exhibiting mildly elevated albuminuria (UACR 30-299 mg/g), microalbuminuria, and group B3 that contained 30 individuals with T2D who have macroalbuminuria, or significantly elevated albuminuria (UACR ≥ 300 mg/g).

Inclusion criteria: As a control group, there were people in good health, people with type 2 diabetes mellitus, people over 40 years, and people of any gender.

Exclusion criteria: The use of drugs known to affect serum FGF21 levels (e.g. fenofibrate), the use of statins, the history of heart failure, alcohol intake, cigarette and tobacco smoking, obesity (BMI ≥ 30 kg/m2), and any indication of macrovascular disease (myocardial infarction, coronary artery disease, peripheral arterial disease, revascularization procedure, coronary artery bypass grafting, and established atherosclerosis) are all linked to ischemia, unstable and stable angina, established atherosclerosis, presence of advanced liver or kidney disease (eGFR ≤ 30), active malignancy, pregnancy and glomerulonephritis.

Ethical clearance: All subjects provided written, informed consent to participate in the research. The Ethical Committee of departments of Internal Medicine and Clinical Biochemistry at Zagazig University Hospitals approved the study, after permission from the Institutional Review Board (IRB) (No. ZU- IRB #: 4195- 6-12-2017). This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All participants were subjected to the following:

I- Complete history taking and clinical examination with particular attention on renal and cardiovascular disease and special stress on measurement of BP, BMI and WC.

II- Routine laboratory investigations:

After putting the whole blood sample at room temperature for two hours or at 2–8 °C for the entire night, centrifuge it for 20 minutes at a weight of around 1000 ×g, and then remove the supernatant. Samples need to be kept cold at -20 °C. Routine laboratory investigations were done according to the protocol of Zagazig University Hospitals (to verify the study respondents' inclusion and exclusion criteria) and included:

1) Complete urine analysis: first-morning sample (for detection of albuminuria, glucose, acetone, pH, bilirubin and leukocytes).
2) Urinary albumin to creatinine ratio (UACR) (mg/g): A morning mid-stream urine sample was collected. Three samples were taken within 3 months and the average value was calculated. Normoalbuminuria was defined as UACR < 30 mg/g, microalbuminuria was defined as UACR 30-299 mg/g and macroalbuminuria was defined as UACR ≥ 300 mg/g [14].
3) Complete blood count.
4) Liver function tests including serum ALT, AST, total and direct bilirubin, albumin, and total protein.
5) Renal function tests including serum urea and creatinine.
6) eGFR by CKD-EP1 2021.
7) PT, PTT, and INR.
8) Fasting glucose, postprandial plasma glucose after two hours and HbA1c.
9) Lipid profile including triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and serum total cholesterol (TC).

III- Other investigations including: ECG, pelviabdominal ultrasound and fundus examination.

1V- Specific investigations including: Serum FGF21 (ng/L):

The serum levels of FGF21 were assessed using the enzyme-linked immunosorbent assay (ELISA). Since FGF21 levels have been shown to exhibit a diurnal pattern, venous blood samples were collected from each participant at 7 or 8 AM after they had fasted for 12 hours the previous night.

Test Principle: (Cat.No.:201-12-1984)

This ELISA kit used the Sandwich-ELISA approach. The micro-ELISA plate that comes with this kit has already been pre-coated with an antibody that was specific to human FGF21. After standards or samples were added to the micro-ELISA plate wells, the matching antibody was added. Next, each microplate well received a sequential addition of a biotinylated detection antibody specific for human FGF21 and an Avidin-Horseradish Peroxidase (HRP). Washing eliminated any free pieces. The substrate solution was poured into each well. Only the wells containing human FGF21, the biotinylated detection antibody, and the Avidin-HRP conjugate were blue in color. The enzyme-substrate reaction was stopped and the color changed to yellow by adding a stop solution. At a wavelength of 450 nm ± 2 nm, the optical density (OD) was measured using spectrophotometry. The human FGF21 levels and the OD value were associated. The concentration of human FGF21 in the samples can be ascertained by comparing their OD to the reference curve.

V- Calculation of 10-years risk of cardiovascular disease using the ACC/AHA ASCVD risk calculator (2013):

Based on the Pooled Cohort Equations, this calculator automatically calculates a 10-year risk for coronary death, nonfatal myocardial infarction, or fatal or nonfatal stroke due to atherosclerotic cardiovascular disease (ASCVD). Age, gender, race, systolic blood pressure, total cholesterol, HDL cholesterol, use of blood pressure-lowering drugs, diabetes, and smoking status are among the details needed to determine their risk of ASCVD. Based on their assessed risk, people are categorized into: 5% is low risk, 5%–7.5% is borderline risk, 7.5-20% is intermediate risk, and ≥ 20% is high risk for the 10-year ASCVD [15].

Statistical analysis

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 20.0. The following tests were used: Chi-square test, independent T test, correlation coefficient, multiple linear regression and ROC curve. $P \leq 0,05$ was considered significant.

RESULTS

There was highly statistically significant increase in SBP & DBP in groups B1, B2, B3 in comparison with group A ($p \leq 0.001$) and statistically significant increase in SBP & DBP in groups B2 and B3 compared to B1($p \leq 0.05$) with no significance difference between groups B2 and B3. There was highly statistically significant increase in serum creatinine in group B3 compared to groups A, B1 & B2 and statistically significant increase in serum creatinine in group B2 as compared to group A. There was a statistically significant increase in mean serum urea level in group B3 compared to groups A, B1 & B2.

There was statistically significant decrease in eGFR in groups B1, B2 & B3 in comparison with group A and highly significant decrease in eGFR in group B3 in comparison with groups B2 & B1 and highly significant decrease in eGFR in group B2 as compared to group B1. There was a highly statistically significant increase in Alb/creatinine ratio in group B3 compared to groups A, B1 & B2 and in group B2 in comparison with groups A & B1 with no significant difference between B1and A. There was a statistically significant increase in total cholesterol, TG, LDL in groups B1, B2 & B3 as compared to group A and significant increase in group B3 compared to groups B1 and B2 with no significant difference between groups B1 and B2.

Regarding HDL, there was highly statistically significant decrease in groups B1, B2 & B3 in comparison with group A and highly statistically significant decrease in group B3 compared to group B1, with no significant difference between groups B1 and B2 & between B2 and B3. There was statistically significant increase in FBG, PPG, HbA1C and duration of diabetes in group B3 in comparison with groups B1 & B2 and statistically significant increase in FBG, PPG, HbA1c and duration of diabetes in group B2 in comparison with group B1 (Table 1).

Table (1): Comparison of demographic, clinical and metabolic parameters among the studied groups

Variable	Group A (N=30)	Group B1 (N=30)	Group B2 (N=30)	Group B3 (N=30)	F-test	P-value
Age (years)	52.6 ± 5.9	50.8 ± 7.2	53.4 ± 4.5	54.9 ± 6.7	2.3	0.083 **(NS)**
SBP (mm Hg)	121 ± 8.2	130.8 ± 11.8 **a**	141.8 ±11.7 **a, b**	146.2 ± 8.3 **a, b**	17.5	**0.000 (HS)**
DBP (mm Hg)	71.2 ±7.2	81.5± 7.6 **a**	87.6 ± 8.8 **a, b**	89.5 ± 9 **a, b**	17.5	**0.000 (HS)**
BMI (Kg/m²)	26.58 ± 2.2	27.48 ± 1.8	27.59 ±1.65	28.01 ± 1.7	1.4	0.258 **(NS)**
W. circum (CM): ***Mean ± SD***	93.3 ± 6.46	95.76 ± 4.8	95.01 ± 5.9	94.06 ± 5.7	1.5	0.219 **(NS)**
Creatinine (mg/dL)	0.79 ±0.18	0.88±0.19	0.94 ± 0.24 **a**	1.1 ± 0.24 **a, b, c**	13.6	**0.000 (HS)**
eGFR (ml/min/1.73m²)	102.4 ± 7.3	95.4 ± 8.4 **a**	78.6 ± 11.7 **a, b**	61.8 ± 10.2 **a, b, c**	108.3	**0.000 (HS)**
Alb/cr ratio (mg/g)	9.3 ±2.4	13± 2.5	165.3 ± 9.6 **a, b**	955.51 ± 9.14**abc**	21.3 (K)	**0.000 (HS)**
Cholesterol (mg/dl)	172.7 ± 16	202.5 ± 32.9 **a**	209.5 ± 34.9 **a**	227.7 ± 36.2 **a, b, c**	16.3	**0.000 (HS)**
TG (mg/dl)	126.5 ± 16.4	156.5 ± 18.7 **a**	155.6 ± 24.2 **a**	177.3 ± 28.8 **a, b, c**	25.7	**0.000 (HS)**
LDL (mg/dl)	95.9 ± 15.3	130.4 ± 34.2 **a**	133.8 ± 31.9 **a**	155.1 ± 39.6 **a, b, c**	18.1	**0.000 (HS)**
HDL (mg/dl)	59.4 ± 7.3	46.6 ± 5.9 **a**	44 ± 5.6 **a**	41.1 ± 5.5 **a, b**	25.7	**0.000 (HS)**
F.B.G (mg/dl)	144.16 ± 28.7	179.7 ± 30.7 **b**	201.1 ± 41.3 **b, c**	201.1 ± 41.3 **b, c**	109	**0.000 (HS)**
PP. G (mg/dl)	203.43 ± 31.6	234.7 ± 37.6 **b**	307.9 ± 61.8 **b, c**	307.9 ± 61.8 **b, c**	99.8	**0.000 (HS)**
HBA1C (%)	7.88 ± 0.78	8.81 ±*0* .96 **b**	9.97 ± 1.5 **b, c**	9.97 ± 1.5 **b, c**	86	**0.000 (HS)**
Duration of DM (years)	7.2 ± 1	10.4 ± 2.7 **b**	14.1± 2.1 **b, c**	14.1 ± 2.1 **b, c**	62.1	**0.000(HS)**
Variable	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**χ 2**	
Sex:										
• ***Males***	13	43.4	14	46.7	15	50	16	53.3	6.8	0.078
• ***Females***	17	56.6	16	53.3	15	50	14	46.7		**(NS)**
HTN:										
• ***No***	30	100	18	60	11	36.7	8	26.7	27.5	**0.000**
• ***Yes***	0	0	12	40	19	63.3	22	73.3		**(HS)**

a = significant in comparison to group A, b = significant in comparison to group B1, c = significant in comparison to group B2

There was highly statistically significant increase in serum FGF21 level in groups B1, B2 & B3 in comparison with group A and significant increase in serum FGF21 level in group B3 compared to groups B1 & B2. Also, significant increase in serum FGF21 in group B2 as compared to group B1. Besides, there was highly statistically significant increase in ASCVD risk in groups B1, B2 & B3 compared to group A and significant increase in group B3 in comparison with groups B1and B2 and significant increase in B2 compared to group B1 (Table 2).

Table (2): Comparison of serum FGF21 level and ASCVD risk among the studied groups

Variable	Group A (N=30)	Group B1 (N=30)	Group B2 (N=30)	Group B3 (N=30)	F-test	P-value
FGF21 (Ng/l): • ***Mean ± SD***	40.13± 4.6	91.4± 8.4 **a**	220.6 ± 9.8 **a, b**	529.48 ± 38.2**a b,c**	91.6 (K)	**0.000 (HS)**
ASCVD risk %: • ***Mean ± S***	1.5 ± 0.4	6.7± 1.8 **a**	12.2 ±2.8 **a, b**	17.9±2 **a, b, c**	21.5 (K)	**0.000 (HS)**

Among the diabetic subgroups (B1, B2 & B3), there was highly statistically significant positive correlation between serum FGF21 level and creatinine, urea, Alb/cr ratio, cholesterol, TG, LDL, FBG, PPG, HBA1c, DM duration and ASCVD. There was a highly statistically significant negative correlation between serum FGF21 level and eGFR & HDL (Table 3).

Table (3): Correlation between serum FGF21 level and other laboratory and clinical measures among diabetic subjects (N=90)

Variable	FGF21	
	r	p-value
Creatinine (mg/dl)	**0.926**	**0.000**
Urea (mg/dl)	**0.955**	**0.000**
eGFR (ml/min/1.73m²)	**-0.885**	**0.000**
Alb/cr ratio (mg/g)	**0.869**	**0.000**
Hb (g/dl)	0.102	0.338
WBCs (x 10³/ul)	0.120	0.260
PLT (x 10³/ul)	0.088	0.408
ALT (IU/L)	0.002	0.986
AST (IU/L)	0.128	0.230
Total protein (g/dl)	0.035	0.0887
Albumin (g/dl)	0.227	0.229
Total bilirubin (g/dl)	0.083	0.435
Cholesterol (mg/dl)	**0.675**	**0.000**
TG (mg/dl)	**0.717**	**0.000**
LDL (mg/dl)	**0.671**	**0.000**
HDL (mg/dl)	**-0.683**	**0.000**
BMI (Kg/m²)	0.0120	0.953
Waist circumference (Cm)	0.067	0.335
SBP (mm Hg)	0.075	0.485
DBP (mm Hg)	0.081	0.354
FBG (mg/dl)	**0.699**	**0.000**
PPG (mg/dl)	**0.917**	**0.000**
HBA1C %	**0.776**	**0.000**
DM duration (years)	**0.516**	**0.000**
ASCVD risk %	**0.547**	**0.000**

Hypertension, serum creatinine, low eGFR, cholesterol, LDL, low HDL, HBA1c, duration of diabetes and FGF21 level were independent predictors for albuminuria in diabetic patients. FGF21 level and duration of diabetes were the best independent predictors (having the highest odd ratio) {O.R (95% C.I), 1.98 (1.54-6.6) with p value, 0.000 for FGF21 and 1.88 (1.36-2.99) with p value, 0.0008 for duration of diabetes} (Table 4).

Table (4): Multivariate logistic regression for prediction of albuminuria among diabetic patients (N=90)

Variable	B	S. E	Wald	O.R (95%C.I)	P-value
HTN (mm Hg)	0.62	0.26	5.63	1.86 (1.11-3.12)	**0.018**
Creatinine(mg/dl)	0.15	0.04	13.8	1.07 (1.07-1.26)	**0.001**
Urea(mg/dl)	0.01	0.12	0.002	0.99 (0.77-1.27)	0.967
eGFR(ml/min/1.73m²)	1.03	0.35	8.67	1.87 (1.31- 4.57)	**0.003**
Total protein (g/dl)	0.04	0.04	0.70	1.04 (0.94-1.14)	0.40
Albumin (g/dl)	0.17	0.14	1.9	1.21 (1.09-1.9)	0.194
Cholesterol(mg/dl)	0.13	0.04	7.6	1.14 (1.03-1.25)	**0.006**
TG (mg/dl)	0.08	0.03	7.7	1.09 (1.02-1.15)	**0.007**
LDL (mg/dl)	0.61	0.30	5.08	1.82 (1.11-3.4)	**0.023**
HDL (mg/dl)	0.65	0.81	12.2	1.84 (1.15-3.8)	**0.000**
FBG (mg/dl)	0.11	0.15	0.54	1.12 (0.82-1.53)	0.45
PPG (mg/dl)	0.37	0.26	2.9	1.45 (0.87-2.4)	0.14
HbA1C %	0.58	0.26	5.04	1.79 (1.07-3)	**0.02**
Duration of DM (years)	0.63	0.16	14.7	1.88 (1.36-2.99)	**0.0008**
ASCVD risk %	0.41	0.30	3.2	1.49 (0.9-1.7)	0.19
FGF21(Ng/L)	0.87	0.91	16.3	1.98 (1.54-6.6)	**0.000**

Table (5) and figure (1) showed ROC curve for serum FGF21 level as an early marker of DKD in type 2 diabetic patients, with sensitivity, specificity, positive predictive value and negative predictive value of 85%, 96.67%, 98.1% and 76.3% respectively and with accuracy of 88.89%.

Table (5): ROC curve for serum FGF21 level as an early marker of DKD in type 2 diabetic patients (N=90)

Cut off	AUC (95%CI)	Sensitivity	Specificity	PPV	NPV	Accuracy	P
>180	0.961 (0.897 to 0.990)	85%	96.67%	98.1%	76.3%	88.89	**<0.0001 ****

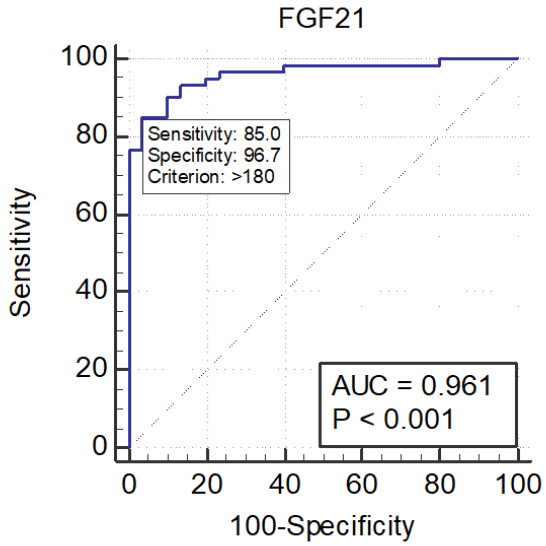

Figure (1): ROC curve for serum FGF21 level as an early marker of DKD in type 2 diabetic patients.

DISCUSSION

The primary cause of renal failure and death for diabetic people is diabetic nephropathy. Many times, microalbuminuria is thought to be a sensitive early indicator of diabetic kidney disease (DKD). Increased cardiovascular morbidity and mortality as well as the onset of atherosclerotic cardiovascular disease are associated with albuminuria [(16)].

We found that there was highly statistically significant increase in ASCVD risk in groups B1, B2 & B3 in comparison with group A and significant increase in group B3 in comparison with groups B1and B2 and significant increase in B2 compared to group B1. Because albuminuria indicates subclinical vascular injury in the kidneys and other vascular beds, it may serve as a predictor of cardiovascular disease risk. Moreover, it might indicate systemic endothelial dysfunction, which increases the risk of subsequent cardiovascular events [(16)]. This correlates with **Ärnlöv and Nowak** [(17)] who investigated the relationship between albuminuria and an adult's risk of cardiovascular outcome during ten years. They concluded that there was a connection between a higher 10-years risk of the cardiovascular event to elevated UACR. When ACR levels were higher than the microalbuminuria threshold, the risk is increased more noticeably.

The FGF21 levels in groups B1, B2, and B3 were significantly higher in our study than in group A. and significant increase in serum FGF21 level in group B3 compared to groups B1 & B2. Also, there was a significant increase in serum FGF21 in group B2 compared to group B1. The body secretes more to protect itself when stress reactions like inflammation and oxidative stress are triggered, which is why serum FGF21 levels are higher in DN patients [(18)]. This goes in agreement with **Jian *et al.*** [(19)] who discovered that serum FGF21 levels were elevated in T2DM patients and significantly higher in individuals in the higher urinary albumin excretion (UAE) group compared to non-diabetic controls. Serum FGF21 levels are independently correlated with UAE in people with type 2 diabetes, which may indicate that circulating FGF21 plays a part in diabetic nephropathy.

Among the diabetic subgroups: (B1, B2 & B3), we found that there was a highly statistically significant positive correlation between the blood level of FGF21 and creatinine, urea, Alb/creatinine ratio, cholesterol, TG, LDL, FBG, PPG, HBA1C, DM duration, and ASCVD. Serum FGF21 level and eGFR & HDL showed a very statistically significant negative correlation. The level of serum FGF21 did not show any significant correlation with any other laboratory markers. Subjects with poor

glucose metabolism may have higher serum levels of FGF21 as a result of FGF21 resistance or as a coping method for enhancing glucose absorption that is inhibited by insulin resistance [20]. This comes in agreement with the results of **Chavez *et al.*** [21] and **Panahi *et al.*** [22] who showed that in comparison with those with well-managed diabetes and healthy controls, those with poorly controlled diabetes had significantly higher serum FGF21 concentrations. Additionally, they discovered a positive correlation between FGF21 levels and glycemia, FPG, 2-h PG, HBA1c, and the length of diabetes.

In contrast, **Cheng *et al.*** [23] discovered that there was no significant differences in serum FGF21 levels between individuals with T2D who received a diagnosis within the last five years and those who had not. Good glycemic control in individuals who had had diabetes over an extended length of time in their study may help to explain this difference. In addition, **Hindricks *et al.*** [24] reported that FGF21, FPG and HBA1c do not correlate. The small sample size and cross-sectional design of their investigation could be the cause of this difference.

The current study revealed that hypertension, serum creatinine, low eGFR, cholesterol, LDL, low HDL, HBA1c, duration of diabetes & FGF21 level were independent predictors for albuminuria in diabetic patients. FGF21 level and duration of diabetes were the best independent predictors (having the highest odd ratio) {O.R (95% C.I), 1.98 (1.54-6.6) with p value, 0.000 for FGF21 and 1.88 (1.36-2.99) with p value, 0.0008 for duration of diabetes}. This correlates with **Esteghamati *et al.*** [25] who found that serum FGF21 and duration of T2D independently predicts microalbuminuria. Furthermore, **Jian *et al.*** [19] found that in diabetic patients, urine albumin excretion was found to be independently correlated with levels of FGF21, FPG, and HDL.

In the current study, serum FGF21 levels, with a cut-off value of 180, may serve as an early marker of DKD for the detection of albuminuria in type 2 diabetes. The marker's sensitivity and specificity were 85% and 96.67% respectively, while, positive and negative predictive values were 98.1% and 76.3%, respectively with an accuracy of 88.89%.

Limitations and recommendations:

The study has certain limitations, such as being a single-center study, therefore the findings may vary at other institutions. Small sample sizes may result in non-significant findings and necessitate further research on the role of current comorbidities. To verify our results and examine variations among various ethnicities, larger sample sizes and research on other populations with diverse ethnic backgrounds are required for future multicenter investigations. More research is required to understand the connection between blood FGF21 levels and metabolic syndrome and to determine whether targeted FGF21 medication therapy can be used to reduce the risks associated with metabolic syndrome.

CONCLUSION:

Prolonged diabetes duration and elevated blood FGF21 levels were the main independent predictors of albuminuria in T2D patients and serum level of FGF21at a certain cut off value could be a marker of early detection of DKD and increased serum level of FGF21 is linked to type 2 diabetes's risk of ASCVD and this will open new therapeutic potential for delaying or prevention of DKD, ASCVD in those patients by using FGF21 antagonist in near future.

Conflict of interest: none declared.
Fund: non-fundable.

REFERENCES

1. **Oguntibeju O (2019):** Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol., 11 (3): 45-63.
2. **Tuttle R, Bakris L, Bilous W *et al.* (2014):** Diabetic kidney disease: a report from an ADA Consensus Conference. Diabetes Care, 37 (10): 2864-2883.
3. **Wang Y, Yuan A, Yu C (2013):** Correlation between microalbuminuria and cardiovascular events. Int J Clin Exp Med., 6 (10): 973-978. **Reigstad J, Varhaug E, Lillehaug R (2005):** Structural and functional specificities of PDGF-C and PDGF-D, the novel members of the platelet-derived growth factors family. FEBS J., 272 (22): 5723-5741.
4. **Zhang F, Yu L, Lin X *et al.* (2015).** Minireview: roles of fibroblast growth factors 19 and 21 in metabolic regulation and chronic diseases. Molecular endocrinology, 29 (10): 1400-1413.
5. **Chang L, Chu C, Huang C *et al.* (2022):** Fibroblast Growth Factor 21 Levels Exhibit the Association with Renal Outcomes in Subjects With Type 2 Diabetes Mellitus. Front. Endocrinol., 13: 846018.
6. **Esteghamati A, Khandan A, Momeni A *et al.* (2017):** Circulating levels of fibroblast growth factor 21 in early-stage diabetic kidney disease. Ir J Med Sci., 186 (3): 785-794.
7. **Jung C , Yoo T (2022):** Pathophysiologic Mechanisms and Potential Biomarkers in Diabetic Kidney Disease. Diabetes Metab J. Mar., 46 (2): 181-197.
8. **Liu Y, Chen Q, Li Y *et al.* (2022):** Advances in FGFs for diabetes care applications. Life Sciences, 310: 121015.
9. **Gallego-Escuredo M, Gómez-Ambrosi J, Catalan V *et al.* (2015):** Opposite alterations in FGF21 and FGF19 levels and disturbed expression of the receptor machinery for endocrine FGFs in obese patients. International journal of obesity, 39 (1): 121-129.
10. **Tantawy N, Sherif E, Matter R *et al.* (2023).** Assessment of fibroblast growth factor 21 in children with type 1 diabetes mellitus in relation to microvascular complications. Pediatric Endocrinology Diabetes and Metabolism, 29 (2): 64-74.
11. **Pippitt K, Li M, Gurgle E (2016):** Diabetes Mellitus: Screening and Diagnosis [published correction appears in

Am Fam Physician., 94 (7): 533]. Am Fam Physician, 93(2):103-109.

12. **Levin A, Stevens E, Bilous W *et al.* (2013):** Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney international supplements, 3 (1): 1-50. 10.1038/ kisup.2012.73
13. **de Carvalho J, Tatsch E, Hausen B *et al.* (2016).** Urinary kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin as indicators of tubular damage in normoalbuminuric patients with type 2 diabetes. Clinical biochemistry, 49 (3): 232-236.
14. **Taherkhani M, Ghasemi M, Taherkhani A (2022):** Predicted ten-year risk of cardiovascular disease among patients without prior heart disease or stroke using atherosclerotic cardiovascular disease risk calculator. Researcher Bulletin of Medical Sciences, 27 (1): e11.
15. **Weir R (2007):** Microalbuminuria and cardiovascular disease. Clin J Am Soc Nephrol., 2 (3): 581-590.
16. **Ärnlöv J, Nowak C (2022):** Association between albuminuria, incident cardiovascular events, and mortality in persons without hypertension, diabetes, and cardiovascular disease. Eur J Prev Cardiol., 29 (1): e4-e6.
17. **Deng J, Liu Y, Liu Y *et al.* (2021):** The Multiple Roles of Fibroblast Growth Factor in Diabetic Nephropathy. J Inflamm Res., 14:5273-5290.
18. **Jian X, Peng H, Jin J *et al.* (2012):** Association between serum fibroblast growth factor 21 and diabetic nephropathy. Metabolism, 61 (6): 853-859.
19. **Lee H, Hui Y, Woo C *et al.* (2015):** Circulating fibroblast growth factor 21 levels predict progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria. J Clin Endocrinol Metab., 100 (4): 1368-1375.
20. **Chavez O, Molina-Carrion M, Abdul-Ghani A *et al.* (2009):** Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care, 32 (8): 1542-1546.
21. **Panahi Y, Bonakdaran S, Yaghoubi A *et al.* (2016):** SERUM LEVELS OF FIBROBLAST GROWTH FACTOR 21 IN TYPE 2 DIABETIC PATIENTS. Acta Endocrinol (Buchar), 12 (3): 257-261.
22. **Cheng X, Zhu B, Jiang F *et al.* (2011):** Serum FGF-21 levels in type 2 diabetic patients. Endocr Res., 36 (4): 142-148.
23. **Hindricks J, Ebert T, Bachmann A *et al.* (2014):** Serum levels of fibroblast growth factor-21 are increased in chronic and acute renal dysfunction. Clin Endocrinol (Oxf)., 80 (6): 918-924.
24. **Esteghamati A, Khandan A, Momeni A *et al.* (2017):** Circulating levels of fibroblast growth factor 21 in early-stage diabetic kidney disease. Ir J Med Sci., 186 (3): 785-794.