ORIGINAL STUDY

Study and Clinical Significance of Urinary Levels of Glypican 5 in Diabetic Patients

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Abstract

Objective: **he objective of this study is to evaluate the urinary glypican5 (GPC5) level in type 2 diabetes mellitus (T2DM) as an early marker for diabetic nephropathy (DN).**

Background: **DN is one of the most serious long-term consequences of T2DM because it causes end-stage renal disease.**

Methods: **This cross-sectional study was done on 90 individuals, classified equally into three groups: group 1: T2DM patients with DN, group 2: T2DM patients without DN and group 3: healthy controls without DM. They underwent [serum creatinine (sCr), serum glycated hemoglobin (HbA1c), urinary albumin to creatinine ratio (ACR), estimated glomerular filtration rate (eGFR), total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), and triglycerides (TG), 2-hour postprandial blood glucose (2h PPG)) and fasting blood glucose (FBG) and urinary GPC5 creatinine ratio] assessment.**

Results: **Systolic blood pressure (SBP), fasting blood glucose, 2-hour postprandial blood glucose, serum glycated hemoglobin, triglycerides, and urinary GPC5 creatinine ratio were significantly elevated in DN group than in the diabetes mellitus (DM) without DN and the controls, and significantly elevated in the DM without DN than in the controls ($P < 0.05$). DN group had a significant elevation in sCr than in the DM without DN and the controls, and a significant decrease in estimated glomerular filtration rate than in the DM without DN and the controls. DN group had a significant elevation in urinary ACR than in the controls ($P2 < 0.001$). Urinary GPC5 creatinine ratio, Urinary ACR, and sCr were significant predictors for developing nephropathy in DM cases.**

Conclusions: **Urinary GPC5 was linked with disease worsening, it could be a good alternative tool to differentiate DN from DM without nephropathy.**

Keywords: Diabetes mellitus, Diabetic nephropathy, Glypican 5, Urinary levels

1. Introduction

Diabetic nephropathy (DN) is a serious long-term consequences of type 2 diabetes mellitus (T2DM) due to its association with end-stage renal disease (ESRD) [1].

Microalbuminuria has been a strong indicator of proteinuria progression. Prior to the development of micro-albuminuria, however, pathological abnormalities have been reported [2].

During renal dysfunction, Glypican5 (GPC5) is expressed in glomerular epithelial cells and excreted in urine [3]. GPC5 urinary concentration may be a useful noninvasive marker for early diagnosis of DN.

The present study aimed to evaluate the urinary GPC5 level in T2DM as an early marker for DN [4].

2. Patients and methods

This cross-sectional study was done on a suitable number of T2DM Egyptian patients and healthy Egyptian people. We enrolled cases aged (18−70), T2DM patients was selected regarding world health organization (WHO) criteria and DN was selected when albumin creatinine ratio (ACR) in urine is within micro-albuminuria levels (30−300 mg/g) or macroalbuminuria levels (>300 mg/g). The study was conducted between 2020 and 2021 with the approval of the university hospital's ethics

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committee. All patients who participated in the research gave their informed consent.

Exclusion criteria were patients with T1DM, coronary heart disease, serious arrhythmia, cerebrovascular disease, bilateral renal artery stenosis, urinary tract infection acute, cardiomyopathy, and severe liver disease.

Cases were classified into three equal groups of people: group 1 : 30 patients of T2DM with DN who were selected from the hospital. Group 2 : 30 patients T2DM without DN who were selected from the hospital. Group 3 (control group): 30 healthy persons without DM who were selected from the hospital.

2.1. All patients underwent

Full history taking as (age, sex, DM and its duration, hypertension (HTN), body mass index (BMI), renal disease, other diseases, and medications), full general examination involving [heart rate, diastolic blood pressure (DBP) and systolic blood pressure (SBP)], full systemic examination including (cardiovascular, abdomen, chest, and neurological examination), fundus examination for detection of diabetes mellitus (DM) retinopathy, renal sonography for excluding obstructive uropathy, to measure the kidneys' size and for the assessment of the parenchymal echogenicity of the kidney, and laboratory investigations including: [blood urea nitrogen, serum creatinine (sCr), serum glycated hemoglobin (HbA1c), ACR in a spot urine sample, glomerular filtration rate (GFR) estimation by the modification of diet in renal disease (MDRD) formula, total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides (TG), 2 h postprandial blood glucose (2h PPG) and fasting blood glucose (FBG) and urinary GPC5 creatinine ratio].

2.2. Kidney ultrasound

The examination was performed by positioning of the patient in supine position, the examiner on his right side, then the probe was placed on the flank with an oblique projection to visualize the kidney in longitudinal axis. The examination of the right kidney was performed through the liver in supine position, with angling the transducer in an oblique angle if liver was small. The scanning position was changed to the right side up decubitus position in case any bowel gas limited the visualizing of the kidney's lower pole, and the kidney was scanned by lateral approach. The patient's left kidney was visualized using a coronal approach through the spleen, with the patient's arm extended over the head and with the patient being positioned in the left side up position. In order to measure bipolar length of the kidney, the suspended inspiration was usually performed by the patient. Renal cortical echogenicity of both kidneys were evaluated by comparing with that of healthy renal sinus and liver, and further classified into: Grade 0: Normal-the cortical echogenicity of the kidney is less than the liver echogenicity. Grade I: the cortical echogenicity of the kidney was equal to the liver echogenicity. Grade II: the cortical echogenicity of the kidney was greater than the liver echogenicity, but less than the renal sinus echogenicity. Grade III: the cortical echogenicity of the kidney is equal to the renal sinus echogenicity.

2.3. Sample collection

To eliminate cell debris, Urine samples were centrifuged at 3000 g at room temperature for 10 minutes. After a 12 h fast, venous blood samples were drawn, and the serum was separated by centrifugation at 4 °C and 4000 g for 10 minutes. Urine and blood samples were stored at −80 °C until analysis. Evaluations were conducted on BP, sCr, HbA1c, and urine ACR.

2.4. Urinary GPC5 concentration determination

Following the manufacturer's instructions, the concentration of GPC5 was measured using an enzyme-linked immunosorbent assay (ELISA) kit purchased from Groundwork Biotechnology Diagnostics (Campanile Drive San Diego, CA, USA). The experiment was conducted in 96-well polystyrene microplates coated with a mouse monoclonal antibody against GPC5. In each well, urine samples and polyclonal antibodies conjugated with horseradish peroxidase against GPC5 were added, mixed, and incubated for 1 hour at 37 °C. In each well, urine samples and polyclonal antibodies conjugated with horseradish peroxidase against GPC5 were added, mixed, and incubated for 1 hour at 37 °C. The GPC5 concentration of each sample was determined using Curve Expert 1.3. We acquired triplicate measurements for each sample. The detection threshold was 0.1 μg/mL, and the GPC5 level was represented as a ratio relative to the urine creatinine concentration.

The medications for controlling blood glucose were sulfonylureas (glimepiride and glibenclamide), pioglitazone, and insulin, and for controlling blood pressure was α-adrenergic and β-adrenergic antagonists, calcium channel blockers, and diuretics, and without ARBs or ACEIs.

2.5. Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) version 25 (IBM Inc., Armonk, NY, USA). Quantitative data distribution was analyzed with the Shapiro–Wilks normality test and histograms to determine whether parametric or nonparametric statistical testing was warranted. The *F* test was used to compare the three groups, and the Tukey post hoc test was used to compare the means and standard deviations of each pair of groups for any parametric variables (such as age) that were reported. The paired *T* test was used to evaluate the relationship between two continuous variables collected from the same set of patients. Categorical variables (like sex) were reported as frequencies and percentages and analyzed statistically using the χ^2 test. In other words, r is the linear correlation coefficient. One group's correlation between two quantitative variables was calculated. Diagnosis performance assessed via sensitivity, specificity, PPV, and NPV (NPV). The factors that were found to be unrelated to the development of the disease were assessed using univariate regression. Statistical significance was assumed when the two-tailed *P* value was less than or equal to 0.05.

3. Results

DBP, TC, LDL was significantly elevated, while HDL was significantly decreased in DN group and the DM group without DN in comparison with the control group ($P < 0.05$). SBP, FBG, 2h PPG, HbA1c, TG, and urinary GPC5 creatinine ratio were significantly elevated in DN group in comparison with the DM group without DN and the control group, and significantly elevated in the DM group without DN in comparison with the controls ($P < 0.05$). DN group had a significant elevation in sCr in comparison with the DM group without DN and the control group, while DN group had a significant decrease in eGFR in comparison with the DM group without DN and the control group. DN group had a significant elevation in urinary ACR in comparison with the control group ($P2 < 0.001$). Incidence of DM retinopathy was significantly higher in DN group and DM group without DN in comparison with the controls ($P < 0.001$). There was insignificant variation regarding sex, age, weight, BMI among the studied groups. DBP, TC, HDL, LDL, and urinary ACR were insignificantly different between DN group and the DM group without DN. Urinary ACR were insignificantly different between DM group without DN and the control group Table 1.

ROC curve between healthy controls and DM without nephropathy reveals a sensitivity of 83% and a specificity of 77%. The ROC curve comparing DM without nephropathy and those with nephropathy reveals a sensitivity of 93.3% and a specificity of 80% Fig. 1.

Urinary GPC5 creatinine ratio had a significant weak positive correlation with SBP, DBP, LDL, and TG, moderate positive correlation with FBG, 2h PPG, HbA1c, and sCr, and strong positive correlation urinary ACR, while it had a strong negative correlation with eGFR. Urinary GPC5 creatinine ratio has no correlation with age, wright, BMI, TC, and HDL Table 2.

SBP, DBP, 2h PPG, FBG, HbA1c, TC, HDL, LDL, TG, sCr, eGFR, urinary ACR and urinary GPC5 creatinine ratio were significant predictors for DN from DM without nephropathy on univariate analysis Table 3.

Table 4 shows that sCr, Urinary ACR and Urinary GPC5 creatinine ratio were significant predictors to developing nephropathy in DM patients among studied variables.

Table 5 shows that FBG, 2h PPG and HbA1c are positive predictors for developing DM.

4. Discussion

Urinary GPC5 is assumed to come from podocytes since podocyte-specific GPC5 knockdown mice showed the lowest detectable urine GPC5 protein concentration in comparison to wild-type mice Okamoto and colleagues [5]. We aimed to study the urinary GPC5 level in T2DM patients as an early marker for DN.

Our investigation revealed that there were statistically significant differences between the DN group and the DM group without nephropathy in terms of FBG, 2h PPG, and HbA1c which were considerably greater in the DN group compared with the DM group without nephropathy, and significantly higher than the control group.

Similar to our findings, Baig [6] discovered that FBG, 2h PPG, and HbA1c were considerably greater in the DM group than in the control group.

Our findings revealed, TC, and LDL were significantly higher in DM groups than control group with no significance between DM groups while HDL was significantly lower in DM groups than control group. Moreover, TG was significantly higher in DM groups than control groups and in DN group than DM group without nephropathy.

In line with our results Palazhy and Viswanathan [7] observed that, TC, TG, LDL-C were significantly higher in DM with nephropathy than in DM without

Table 1. Laboratory variables in participants of different groups.

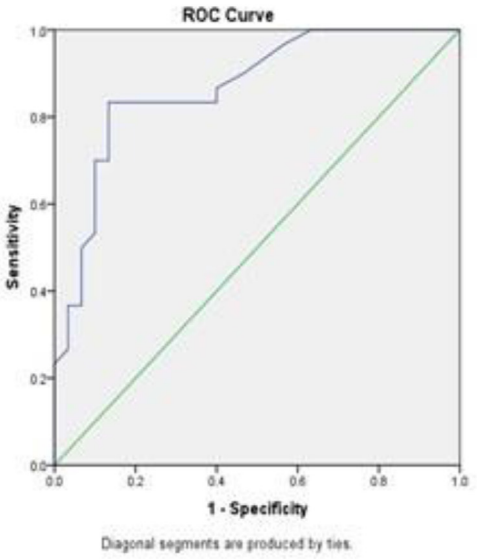
Laboratory variables	Group I diabetic nephropathy ($n = 30$)	Group II diabetic without nephropathy ($n = 30$)	Group III control ($n = 30$)	Test of significance	*P* value	
Sex						
Male	12(40.0%)	14(46.7%)	17(56.7%)	1.692 Chi square test	0.429	
Female	18(60.0%)	16(53.3%)	13(43.3%)			
Age	59.33 ± 7.95	58.93 ± 10.47	56.20 ± 8.66	1.06 ANOVA	0.352	
Weight in kg	79.83 ± 9.27	75.97 ± 9.00	74.70 ± 7.82	2.82 ANOVA	0.065	$P1 = 0.235$ $P2 = 0.080$ $P3 = 0.854$
BMI (kg/m^2)	27.03 ± 3.11	26.63 ± 2.88	23.37 ± 2.69	1.97 ANOVA	0.145	$P1 = 0.877$ $P2 = 0.165$ $P3 = 0.376$
SBP (mmHg)	142.20 ± 12.45	134.50 ± 7.86	127.83 ± 7.59	16.94 ANOVA	<0.001[a]	$P1 = 0.01$[a] $P2<0.001$[a] $P3 = 0.030$[a]
DBP (mmHg)	85.40 ± 12.03	80.43 ± 7.06	72.70 ± 5.84	16.13 ANOVA	<0.001[a]	$P1 = 0.094$ $P2<0.001$[a] $P3 = 0.004$[a]
Diabetic retinopathy						
Absent	8(26.7%)	23(76.7%)	30(100.0%)	38.56	<0.001[a]	
Present	22(73.3%)	7(23.3%)	0(0.0%)			
FBG (mg/dl)	215.90 ± 47.76	173.10 ± 34.37	86.97 ± 8.38	109.87	<0.001[a]	$P1<0.001$[a] $P2<0.001$[a] $P3<0.001$[a]
2h Post prandial blood glucose (mg/dl)	299.90 ± 60.29	244.20 ± 39.36	123.07 ± 11.75	138.22	<0.001[a]	$P1<0.001$[a] $P2<0.001$[a] $P3<0.001$[a]
HbA1c	9.83 ± 1.37	8.17 ± 1.25	5.51 ± 0.24	121.72	<0.001[a]	$P1<0.001$[a] $P2<0.001$[a] $P3<0.001$[a]
TC mmol/l	240.30 ± 45.74	239.47 ± 43.29	183.27 ± 24.72	21.01	<0.001[a]	$P1 = 0.997$ $P2<0.001$[a] $P3<0.001$[a]
HDL (mg/dl)	41.70 ± 7.02	42.17 ± 4.94	50.23 ± 10.11	11.77	<0.001[a]	$P1 = 0.973$ $P2<0.001$[a] $P3<0.001$[a]
LDL (mg/dl)	137.30 ± 30.93	136.13 ± 28.65	102.37 ± 14.09	17.54	<0.001[a]	$P1 = 0.985$ $P2<0.001$[a] $P3<0.001$[a]
TG (mg/dl)	274.47 ± 104.32	211.17 ± 90.17	128.20 ± 26.79	24.55	<0.001[a]	$P1 = 0.013$[a] $P2<0.001$[a] $P3<0.001$[a]
Serum creatinine (mg/dl)	1.50 ± 0.82	0.72 ± 0.09	0.70 ± 0.11	27.27	<0.001[a]	$P1<0.001$[a] $P2<0.001$[a] $P3 = 0.988$
eGFR (mL/min 1.73m2)	60.30 ± 33.17	103.23 ± 13.98	111.57 ± 15.84	44.02	<0.001[a]	$P1<0.001$[a] $P2<0.001$[a] $P3 = 0.369$
Urinary Albumin creatinine ratio (mg/g)	1006.30 ± 924.532	21.27 ± 5.62	11.17 ± 4.25	34.41	<0.001[a]	$P1 = 0.997$ $P2<0.001$[a] $P3 = 0.997$
Urinary Glypican-5 creatinine ratio (μg/g)	2.66 ± 0.78	1.35 ± 0.29	1.01 ± 0.17	93.21	<0.001[a]	$P1<0.001$[a] $P2<0.001$[a] $P3 = 0.035$[a]

Data are presented as mean ± SD or frequency (%).

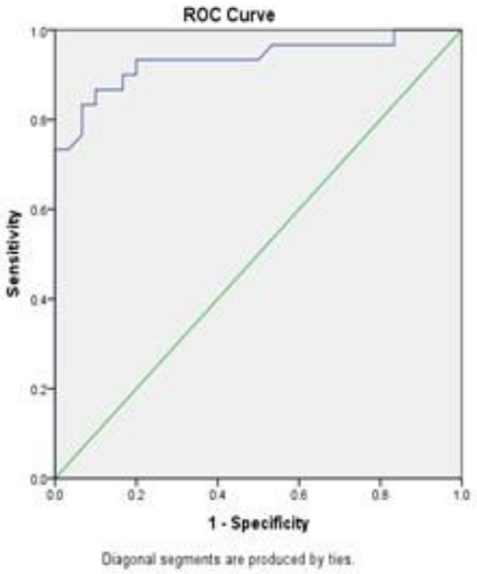
BMI, body mass index; DBP, diastolic blood pressure; DN, Diabetic nephropathy; eGFR, Estimated glomerular filtration rate; FBG, Fasting blood glucose; HbA1c, Glycosylated hemoglobin; HDL, High density lipoproteins; LDL, Low density lipoproteins; SBP, systolic blood pressure; TC, Total cholesterol; TG, Triglycerides.

*P*1 between diabetic nephropathy and diabetic group without nephropathy, *P*2 between diabetic nephropathy and control, *P*3 between diabetic group without nephropathy and control.

[a] Significant as P value < 0.05.



(a)



(b)

Fig. 1. Roc curve of Urinary GPC5 cr. ratio (μg/g) between (a) diabetic group without nephropathy and controls, (b) diabetic group without nephropathy and diabetic nephropathy group.

nephropathy with no difference between both groups as regard HDL. This came in contrast with Li and colleagues who found that, TG, LDL, and HDL was no significantly different between DM groups and control group as regard except in cholesterol results that came in line with our results.

Our study showed that, sCr, and urinary ACR were significantly higher in DN cases than DM subjects without nephropathy, while eGFR was significantly lower in DN cases than both subjects without nephropathy and controls.

Also, Li and colleagues [8] discovered that sCr was considerably greater in the DN group than the DM group. eGFR was lower in the DN group than in the DM group.

Results from studies on cases with T2DM have shown that micro- and macroalbuminuria at baseline, as well as rising albuminuria, pose greater risks of declining kidney function and associated outcomes than do currently available renal biomarkers Norris and colleagues [9].

In a meta-analysis of five trials including patients with T2DM, individuals with micro-albuminuria had a roughly 4-fold (95 %CI: 1.6−8.4) higher RR for developing ESRD than those with normo-albuminuria. Absolute variations in albuminuria were also evaluated as predictors of renal disease progression in T2DM cases Macisaac and colleagues [10].

Regarding our findings, urinary GPC5 creatinine ratio there was significant difference between DN and DM group without nephropathy (*P* value < 0.001), between DN and control group and between DM group without nephropathy and control, that came in line with Li and colleagues who found that urinary GPC5 was higher in DN than in DM cases and healthy controls.

The GPC5 level was substantially greater in the DN group than in the DM and healthy control

Table 2. Correlation of Urinary GPC5 creatinine ratio and different parameters.

Different parameters	Correlation coefficient (r)	*P* value
Age (years)	0.168	0.114
Weight (kg)	0.148	0.165
BMI (kg/m^2)	0.088	0.407
SBP (mmHg)	0.386	<0.001[a]
DBP (mmHg)	0.360	<0.001[a]
FBG (mg/dl)	0.570	<0.001[a]
2h Post prandial blood glucose (mg/dl)	0.612	<0.001[a]
HbA1c	0.586	<0.001[a]
TC mmol/l	0.18	0.090
HDL (mg/dl)	−0.20	0.058
LDL (mg/dl)	0.268	0.011[a]
TG (mg/dl)	0.232	0.028[a]
Serum creatinine (mg/dl)	0.675	<0.001[a]
eGFR (mL/min1.73m^2)	−0.732	<0.001[a]
Urinary Albumin creatinine ratio (mg/g)	0.712	<0.001[a]

BMI, Body mass index; DBP, Diastolic blood pressure; eGFR, Estimated glomerular filtration rate; FBG, Fasting blood glucose; HbA1c, Glycosylated hemoglobin; HDL, High density lipoproteins; LDL, Low density lipoproteins; SBP, Systolic blood pressure; TC, Total cholesterol; TG, Triglycerides.

[a] Significant as P value < 0.05.

Table 3. Univariate analysis between DN and DM without nephropathy.

Variables	Standardized β	Standard. Error	*P* value	95% CI	
				Lower	Upper
Age	0.82	0.1698	0.468	0.490	1.392
Body weight in kg	1.28	1.668	. 0.143	0.92	1.79
Body mass index	0.92	0.548	0.707	0.61	1.40
SBP	1.422	0.1901	<0.001[a]	1.383	1.460
DBP	8.540	1.800	<0.001[a]	8.179	8.900
FBG	2.159	7.596	<0.001[a]	2.006	2.311
2h Post prandial blood glucose	2.999	9.295	<0.001[a]	2.812	3.185
HbA1c	9.831	0.240	<0.001[a]	9.351	10.311
TC mmol/l	2.403	8.130	<0.001[a]	2.240	2.565
HDL (mg/dl)	4.170	1.109	<0.001[a]	3.948	4.391
LDL (mg/dl)	1.373	0.544	<0.001[a]	1.264	1.481
TG (mg/dl)	2.711	1.728	<0.001[a]	2.365	3.057
Serum creatinine (mg/dl)	1.504	0.107	<0.001[a]	1.291	1.717
Estimated Glomerular Filtration Rate (mL/min1.73m^2)	6.030	4.648	<0.001[a]	5.099	6.960
Urinary Albumin creatinine ratio (mg/g)	10.060	11.93	<0.001[a]	7.673	12.452
Urinary Glypican-5 creatinine ratio (μg/g)	2.662	0.108	<0.001[a]	2.445	2.879

CI, confidence interval; DBP, Diastolic blood pressure; FBG, Fasting blood glucose; HbA1c, Glycosylated hemoglobin; HDL, High density lipoproteins; LDL, Low density lipoproteins; SBP, Systolic blood pressure; TC, Total cholesterol; TG, Triglycerides.
[a] Significant as P value < 0.05.

groups ($P < 0.001$). The levels of GPC5 in the DM group (1.55 0.46 ng/g) and the healthy controls (1.29 0.33) did not vary substantially. The concentration of GPC5 in spot urine was determined by both the amount of GPC5 released by podocytes and the urine flow rate.

The presence of DM retinopathy on fundus examination was significantly different among the three groups and being higher in DN group with 73.3%. In contrast with our results, Palazhy and Viswanathan reported that retinopathy was insignificantly different between both DN group and DM group [7].

Urinary GPC5 creatinine ratio in one side was significantly positive correlated with SBP, DBP, FBG, 2h PPG, HbA1c, LDL, TG, sCr and Urinary

Table 4. Predictors of DN among diabetics using binary logistic regression.

Parameters	*P* value	Odd's ratio	95% CI	
			Lower	Upper
SBP	0.479	0.952	0.832	1.090
DBP	0.583	1.039	0.907	1.189
FBG	0.807	0.993	0.940	1.050
2h Post prandial blood glucose	0.485	1.011	0.980	1.044
HbA1c	0.459	0.581	0.138	2.444
TC mmol/l	0.829	1.006	0.951	1.065
HDL (mg/dl)	0.714	0.967	0.810	1.155
LDL (mg/dl)	0.919	1.005	0.918	1.099
TG (mg/dl)	0.325	0.994	0.983	1.006
Serum creatinine (mg/dl)	0.035*	2.608	2.184	3.056
eGFR (mL/min1.73m^2)	0.371	1.034	0.961	1.111
Urinary Albumin creatinine ratio (mg/g)	0.046*	1.990	1.997	2.002
Urinary Glypican-5 creatinine ratio (μg/g)	0.009*	2.714	2.139	3.445

Significant as *P* value < 0.05.
CI, confidence interval; DBP, Diastolic blood pressure; eGFR, Estimated glomerular filtration rate; FBG, Fasting blood glucose; HbA1c, Glycosylated hemoglobin; HDL, High density lipoproteins; LDL, Low density lipoproteins; SBP, Systolic blood pressure; TC, Total cholesterol; TG, Triglycerides.

Table 5. Binary logistic regression analysis between diabetic group without nephropathy and control group.

Parameters	*P* value	Odd's ratio	95% CI	
			Lower	Upper
SBP	0.914	0.992	0.854	1.152
DBP	0.744	1.029	0.867	1.220
FBG	0.013[a]	1.758	1.019	3.033
2h Post prandial blood glucose	0.017[a]	1.860	1.670	2.075
HbA1c	0.021[a]	2.023	1.883	2.175
TC mmol/l	0.808	1.005	0.963	1.050
HDL (mg/dl)	0.863	0.990	0.881	1.112
LDL (mg/dl)	0.861	0.993	0.922	1.070
TG (mg/dl)	0.783	1.002	0.987	1.018
Serum creatinine (mg/dl)	0.871	1.303	0.873	1.946
eGFR (mL/min1.73m^2)	0.929	0.997	0.934	1.065
Urinary Albumin creatinine ratio (mg/g)	0.492	1.054	0.907	1.225
Urinary Glypican-5 creatinine ratio (μg/g)	0.810	1.118	0.794	1.576

CI, confidence interval; DBP, Diastolic blood pressure; eGFR, Estimated glomerular filtration rate; FBG, Fasting blood glucose; HbA1c, Glycosylated hemoglobin; HDL, High density lipoproteins; LDL, Low density lipoproteins; SBP, Systolic blood pressure; TC, Total cholesterol; TG, Triglycerides.
[a] Significant as P value < 0.05.

ACR on the other side. Urinary GPC5 creatinine ratio was significantly negative correlated with eGFR. Similarly with our findings, Li and colleagues [8] reported that the change in the GPC5 level were significantly negative correlated with eGFR, r = −0.786, $P < 0.001$), and the change in the GPC5 level were significantly positive correlated with 24 h urine protein (r = 0.33, $P = 0.046$) and albumin (r = 0.346, $P = 0.027$) excretion.

Our findings showed that, urinary GPC5 creatinine ratio is a good alternative diagnostic tool to differentiate between DN group and DM group without nephropathy. ROC curve between controls and DM without nephropathy shows that the sensitivity was 83% while specificity was 77%, while Roc curve between DM without nephropathy and DN group shows that the sensitivity was 93.3% while specificity was 80%.

There was a significant difference between normal and macroalbuminuria group regarding level of sCr, eGFR and Urinary GPC5 cr. Ratio. there was significant difference between micro-albuminuria and macroalbuminuria group regarding level of sCr, eGFR (mL/min 1.73 m^2) and urinary GPC5 cr. Ratio. This came in line with Xie and colleagues [11] found that there was a significant difference among three groups as regard eGFR.

Podocyte damage caused by exposure to hyperglycaemic filtrate may explain for the presence of GPC5 in urine and the loss of podocyte markers, including GPC5 Weil and colleagues [12].

Our results found that, urinary ACR in one hand was significantly positive correlated with sCr and urinary GPC5 cr. ratio. Also, there between Urinary ACR was significantly negative correlated with eGFR.

In the same context, Ibrahim and colleagues observed that, there was a significant difference in normoalbuminuric group in FBS, HA1c, BP and GFR when compared with normal group and no significant difference between both groups in ACR and sCr. There was a significant difference in macroalbuminuric group in FBS, HbA1c, BP, GFR, ACR and sCr in comparison with normal group. There was a significant difference in macroalbuminuric group in GFR and ACR than normoalbuminuric group [13].

In Li and colleagues series, the high urine GPC5 levels at presentation were related with not only an increase in urinary protein and albumin excretion, but also a decrease in eGFR. Urine GPC5 levels predicted the development of DN sooner than urinary albumin and protein levels [8].

There was a significant difference between DN group and DM group without nephropathy regarding SBP, DBP, FBG, 2h PPG, HbA1c, TC, HDL, LDL, TG, sCr, eGFR, Urinary ACR and Urinary GPC5 creatinine ratio on univariate analysis.

Also, Abougalambou *and colleague*s [14] studied 1077 T2DM and found that, the creatinine clearance rate was substantially different between individuals with and without nephropathy, and proteinuria was related with the nephropathy group.

Our findings observed sCr, urinary ACR and Urinary GPC5 creatinine ratio were significant predictors to developing nephropathy in DM patients among studied variables.

Also, Hu and Zhang [15] found that HTN (OR = 1.768, $P = 0.042$), hyperuricemia (OR = 2.263, $P = 0.003$), SBP (OR = 1.027, $P < 0.001$), and HbA1c (OR = 1.358, $P < 0.001$) were risk factors for DN in T2DM patients on binary logistic regression analysis.

FBG, 2h PPG, and HbA1c are good indicators for the advancement of DM complications, according to our data. Earlier research indicated that PPG has a strong connection with HbA1c or significantly contributes to glycaemic management overall. Recent studies have shown a strong link between PPG and the development of DM complications, therefore this is consistent with those findings Ketema and Kibret [16].

Our research had several limitations, including a small sample size, a single-centre design, a short follow-up period, and a single test of kidney function during analysis.

4.1. Conclusions

Urinary GPC5 was specifically higher in T2DM cases with DN, and it was related with disease development. Among the examined variables, urinary ACR (mg/g) and Urinary GPC5 creatinine ratio were significant predictors of developing nephropathy in DM individuals. FBG, 2h PPG, and HbA1c are predictors of the development of DM problems.

Urinary GPC5 creatinine ratio is a good alternative diagnostic tool to differentiate between DN group and DM group without nephropathy with sensitivity was 93.3% while specificity was 80%. Further larger cohort clinical studies are needed with multicentre cooperation to validate our findings. Longer duration of follow up is needed for additional investigation.

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Nil.

Presentation at a Meeting

No.

Conflicts of interest

No conflict of interest.

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