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RESEARCH ARTICLE OPEN ACCESS

Urinary glypican-5 concentration as an early associated biomarker for diabetic nephropathy

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Abstract
Objective: Early diagnosis of diabetic nephropathy (DN) is limited by the low sensitivity of albuminuria and estimated glomerular filtration rate (eGFR). Urinary glypican-5 (GPC5), a podocyte heparan sulfate proteoglycan, is a candidate biomarker. This cross-sectional study evaluated the association between urinary GPC5 and DN severity in type 2 diabetes mellitus (T2DM).
Methods: We enrolled 100 Iraqi T2DM patients (65 male, 35 female; mean age 58.3 years) and 50 healthy controls (32 male, 18 female; mean age 54.6 years). Fasting blood glucose, HbA1c, urine albumin-to-creatinine ratio (UACR), eGFR (CKD-EPI), and urinary GPC5 (ELISA) were measured. Patients were stratified by albuminuria (normo-/micro-/macro-albuminuria) and eGFR stages (G1–G4). Analyses used t-tests, ANOVA with Tukey post-hoc, and Pearson correlation.
Results: Urinary GPC5 was higher in patients than controls (1.97±0.45 vs. 1.17±0.25 ng/mL, p<0.001). GPC5 was already elevated in normoalbuminuric patients (1.62±0.32 ng/mL) and increased across micro-albuminuric (1.92±0.36) and macroalbuminuric (2.32±0.40) groups (p<0.001 for trend). Similarly, GPC5 rose with declining eGFR: normal (1.54±0.30), mild (1.84±0.28), moderate (1.98±0.35), severe (2.27±0.43 ng/mL; all p<0.001 vs. controls). GPC5 correlated positively with HbA1c (r=0.68, p<0.001) and negatively with eGFR (r=−0.76, p<0.001).
Conclusions: Urinary GPC5 is strongly associated with DN presence and severity, including in normoalbuminuric patients. These findings support further evaluation of GPC5 as an early diagnostic biomarker, but prospective validation is needed.

Keywords: Diabetic nephropathy, glypican-5, albuminuria, glomerular filtration rate, early diagnosis

Plain English Summary

Diabetic kidney disease is a common complication of diabetes that can lead to kidney failure. Current urine tests detect kidney damage only after significant injury has occurred. This study examined whether a protein called glypican-5, which is released from kidney cells, appears in the urine of people with diabetes earlier than standard tests. We measured glypican-5 in urine samples from 100 diabetic patients and 50 healthy people. We found that glypican-5 levels were higher in diabetic patients, and importantly, they were already elevated even in those whose standard kidney tests were still normal. Levels increased as kidney disease worsened. This suggests that measuring urinary glypican-5 might help doctors detect diabetic kidney disease earlier, giving a chance to start protective treatments sooner. However, larger studies that follow patients over time are needed to confirm this finding.

Introduction

Diabetic nephropathy (DN) is one of the most serious complications of long-standing, uncontrolled diabetes mellitus, and it remains the leading cause of end-stage kidney disease worldwide. Approximately 30–40% of patients with diabetes develop DN during their lifetime (1). The classic clinical hallmark of early DN is

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microalbuminuria (urine albumin-to-creatinine ratio [UACR] 30–300 mg/g), which predicts progression to overt nephropathy and increased cardiovascular risk. However, it is now well recognised that significant structural and functional kidney damage can begin even before microalbuminuria becomes detectable (2). Moreover, up to 25–23% of type 2 diabetes patients with DN may not have overt proteinuria, highlighting the limited sensitivity of albuminuria alone (3).

The estimated glomerular filtration rate (eGFR) complements UACR in staging chronic kidney disease, but it also lacks sensitivity for very early tubular and glomerular injury (4). Both markers vary among populations and can be influenced by non-diabetic factors such as hydration, infection, and exercise (4, 6). Consequently, there is a pressing need for more sensitive and specific urinary biomarkers that can detect incipient DN before irreversible damage occurs. Tubular injury, as reflected by tubular injury markers, is now recognised as an early component of DN, and such markers may be elevated even in normoalbuminuric patients (7, 8, 9, 10, 11).

Glypican-5 (GPC5) is a cell-surface heparan sulfate proteoglycan expressed on podocytes and glomerular mesenchymal cells. Genome-wide association studies have linked a variant in the GPC5 gene to acquired nephrotic syndrome and to type 2 diabetes (12, 13). In diabetic conditions, hyperglycaemia upregulates GPC5 expression in mesangial cells and podocytes, and the protein is shed into urine. GPC5 can bind and enhance basic fibroblast growth factor (FGF2) signalling, which alters podocyte differentiation and increases glomerular albumin permeability (14). Experimental studies in mice have shown that podocyte-specific GPC5 knockout reduces urinary GPC5 levels, confirming its podocyte origin (15). Recent work suggests that urinary GPC5 correlates with the severity of DN, but data from Middle Eastern populations remain scarce.

In this study, we aimed to evaluate the association between urinary GPC5 concentration and the presence and severity of DN in Iraqi patients with type 2 diabetes. We also explored whether GPC5 is elevated in normoalbuminuric patients, which would support its potential as an early diagnostic biomarker.

Materials and Methods

Study design and setting

This cross-sectional study was conducted at the Consulting Clinic of Azadi Hospital in Kirkuk city, Iraq, from October 2024 to January 2025. The study protocol was approved by the Scientific Committee of the College of Medicine, University of Tikrit (approval reference: TU/COM/2024/143, dated 15 September 2024). All procedures followed the ethical standards of the institutional research committee and the 1964 Helsinki declaration and its later amendments.

Participants

A total of 150 participants were enrolled: 100 patients with type 2 diabetes mellitus (T2DM) and 50 healthy controls. Diabetic patients were recruited consecutively from the outpatient clinic. Inclusion criteria were: diagnosis of T2DM for at least two years, age between 45 and 75 years, and willingness to provide informed consent. Exclusion criteria included: known non-diabetic kidney disease (e.g., glomerulonephritis, polycystic kidney disease), acute kidney injury in the preceding three months, urinary tract infection, pregnancy, malignancy, or use of nephrotoxic drugs (other than ACE inhibitors/ARBs). Patients already on regular dialysis (stage G5) were excluded because dialysis alters urinary biomarker levels.

The control group comprised 50 healthy volunteers recruited from the same geographic area, matched for age and sex, with no history of diabetes, hypertension, or kidney disease, and with normal urinalysis and eGFR ≥ 90 mL/min/1.73 m².

All participants provided written informed consent before enrolment.

Data collection and clinical measurements

A structured questionnaire captured demographic data, diabetes duration, and medication history. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Blood pressure was measured twice after five minutes of rest using a standard mercury sphygmomanometer.

Laboratory methods

Blood sampling: Approximately 6 mL of venous blood was collected from each participant after an overnight fast. Whole blood was used immediately for HbA1c measurement using an automated cation-exchange high-performance liquid chromatography method (Bio-Rad D-10, Hercules, CA, USA). Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at –80°C until analysis. Fasting blood glucose (FBG) and serum creatinine were measured using a clinical biochemistry analyser (RX Daytona, Randox Laboratories, UK). eGFR was calculated using the CKD-EPI 2021 equation without the race coefficient.

Urine sampling: A mid-stream morning urine sample (25 mL) was collected from each participant. Samples were centrifuged at 2000 rpm for 10 minutes to remove cellular debris. The

supernatant was aliquoted and stored at –80°C. Urine albumin and creatinine were measured by immunoturbidimetry and the Jaffe method, respectively, and UACR was calculated (mg/g). For glypican-5 measurement, urine samples were thawed and analysed in duplicate using a commercial sandwich enzyme-linked immunosorbent assay (Human Glypican-5 ELISA Kit, catalogue No. E-EL-H5555, Elabscience, Wuhan, China) according to the manufacturer's instructions. The assay had a detection range of 0.16–10 ng/mL, with intra-- and inter-assay coefficients of variation < 8% and < 10%, respectively. Results are expressed as ng/mL of urine.

Classification of diabetic nephropathy severity

Diabetic patients were categorised according to two complementary systems:

By albuminuria status (based on UACR from three samples over three months, but a single measurement was used for this cross-sectional analysis):

1. Normoalbuminuria: UACR < 30 mg/g (n = 27)
2, Microalbuminuria: UACR 30–300 mg/g (n = 41)
3. Macroalbuminuria: UACR > 300 mg/g (n = 32)

By eGFR stage (KDIGO classification, mL/min/1.73 m²):

1. G1 (normal): ≥ 90 (n = 20)
2. G2 (mild): 60–< 90 (n = 16)
3. G3 (moderate): 30–< 60 (n = 30)
4. G4 (severe): 15–< 30 (n = 34)
5. G5 (kidney failure, < 15 or dialysis) was excluded.

Statistical analysis

Data were analysed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality using the Shapiro–Wilk test. All variables were normally distributed, so parametric tests were used. Data are presented as mean ± standard deviation (SD). Comparisons between two groups were performed using an independent Student's t-test, and comparisons among three or more groups used one-way ANOVA followed by Tukey's honest significant difference (HSD) post-hoc test for multiple comparisons. Pearson's correlation coefficient was used to assess linear relationships between urinary GPC5 and other continuous variables. A p-value < 0.05 (two-tailed) was considered statistically significant. No adjustment for multiple comparisons was applied to the primary correlations because they were prespecified; however, post-hoc comparisons were adjusted using Tukey's method.

Sample size was not calculated a priori; the study included all eligible patients who consented during the recruitment period. No data were missing.

Results

Demographic and clinical characteristics of the study population

The study included 100 diabetic patients (65 males, 35 females) and 50 healthy controls (32 males, 18 females). The mean age was comparable between patients and controls (58.3 ± 8.4 vs. 54.6 ± 8.8 years, p = 0.53), confirming adequate age matching. However, among diabetic patients, females were significantly older than males (61.5 ± 7.6 vs. 56.6 ± 8.4 years, p = 0.005). This is consistent with the later onset of type 2 diabetes in women in this population.

As expected, diabetic patients had significantly higher FBG (147.1 ± 28.8 vs. 93.5 ± 10.9 mg/dL, p < 0.001), HbA1c (7.47 ± 0.77 vs. 5.70 ± 0.47%, p < 0.001), and lower eGFR (53.5 ± 30.0 vs. 101.0 ± 5.8 mL/min/1.73 m², p < 0.001) compared to controls. Interestingly, BMI was slightly lower in patients than in controls (24.8 ± 3.8 vs. 26.3 ± 4.1 kg/m², p = 0.023). This unexpected finding may reflect a survivor or selection effect, as patients with long-standing diabetes sometimes experience weight loss, or it may be due to chance.

Within the diabetic group, there were no significant differences between males and females in FBG, HbA1c, eGFR, BMI, or urinary GPC5 (all p > 0.12, Table 1), indicating that sex did not confound the primary analyses.

Table 1: Clinical and laboratory parameters in male and female diabetic patients (n = 100)

Variable	Male (n=65)	Female (n=35)	p-value
FBG (mg/dL)	128.4 ± 33.2	130.8 ± 38.3	0.23
HbA1c (%)	6.99 ± 1.14	6.70 ± 0.95	0.68
eGFR (mL/min/1.73 m²)	67.3 ± 33.8	72.7 ± 32.8	0.12
BMI (kg/m²)	25.3 ± 4.1	25.3 ± 3.7	0.92
Urinary GPC5 (ng/mL)	1.70 ± 0.56	1.70 ± 0.52	0.99

Data are mean ± SD. No significant differences between sexes

Urinary glypican-5 is elevated in diabetic patients and increases with disease severity

Urinary GPC5 concentration was significantly higher in diabetic patients than in healthy controls (1.97 ± 0.45 vs. 1.17 ± 0.25 ng/mL, p < 0.001).

This difference was consistent across all subgroups.

Association with albuminuria categories: As shown in Table 2 and Figure 1, urinary GPC5 increased progressively across the three albuminuria groups. Importantly, even normoalbuminuric patients had significantly higher GPC5 levels than controls (1.62 ± 0.32 vs. 1.17 ± 0.25 ng/mL, p < 0.001). GPC5 was further elevated in microalbuminuric (1.92 ± 0.36 ng/mL) and macroalbuminuric (2.32 ± 0.40 ng/mL) patients. All pairwise comparisons between groups were statistically significant (p < 0.001), except that the difference between normo- and microalbuminuria did not reach significance in the post-hoc analysis (p = 0.08), possibly due to sample size. Nevertheless, the overall trend was highly significant (ANOVA p < 0.001).

Table 2: Urinary glypican-5 levels across albuminuria categories

Group	n	Urinary GPC5 (ng/mL), mean ± SD
Healthy controls	50	1.17 ± 0.25
Normoalbuminuria	27	1.62 ± 0.32*
Microalbuminuria	41	1.92 ± 0.36*†
Macroalbuminuria	32	2.32 ± 0.40*†‡
Total	150	1.70 ± 0.54

*ANOVA p < 0.001. *p < 0.001 vs. controls; †p < 0.05 vs. normoalbuminuria; ‡p < 0.05 vs. microalbuminuria (Tukey post-hoc)*

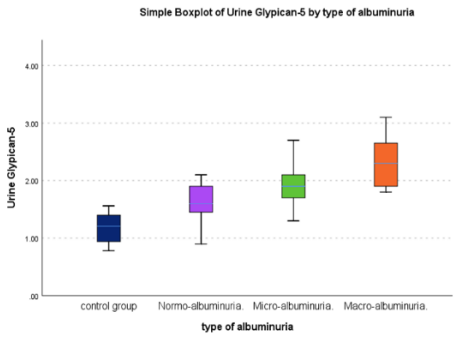


Figure 1: Urinary glypican-5 concentration by albuminuria status

Association with eGFR stages: Similarly, urinary GPC5 rose significantly with declining eGFR (Table 3). Patients with normal eGFR (G1) already had higher GPC5 than controls (1.54 ± 0.30 vs. 1.17 ± 0.25 ng/mL, p < 0.001). Levels increased further in G2 (1.84 ± 0.28), G3 (1.98 ± 0.35), and G4 (2.27 ± 0.43 ng/mL). The overall ANOVA was highly significant (p < 0.001), and post-hoc comparisons showed that each stage differed from controls and from the most severe stage (G4), though differences between adjacent stages (e.g., G2 vs. G3) were not always significant (Table 4).

Table 3: Urinary glypican-5 levels across eGFR stages (KDIGO)

eGFR stage	n	Urinary GPC5 (ng/mL), mean ± SD
Controls (eGFR ≥90)	50	1.17 ± 0.25
G1 (≥90)	20	1.54 ± 0.30*
G2 (60–<90)	16	1.84 ± 0.28*†
G3 (30–<60)	30	1.98 ± 0.35*†
G4 (15–<30)	34	2.27 ± 0.43*†‡
Total	150	1.70 ± 0.54

*ANOVA p < 0.001. *p < 0.001 vs. controls; †p < 0.05 vs. G1; ‡p < 0.05 vs. G2 and G3*

Table 4: Pairwise comparisons of urinary GPC5 between eGFR stages (post-hoc Tukey)

Comparison	Mean difference (ng/mL)	p-value
Controls vs. G1	–0.366	<0.001
Controls vs. G2	–0.670	<0.001
Controls vs. G3	–0.803	<0.001
Controls vs. G4	–1.100	<0.001
G1 vs. G2	–0.304	0.051
G1 vs. G3	–0.437	<0.001
G1 vs. G4	–0.734	<0.001

G2 vs. G3	–0.133	0.688
G2 vs. G4	–0.430	<0.001
G3 vs. G4	–0.297	0.004

Only significant differences ($p < 0.05$) are highlighted in the text; the Tukey method accounts for multiple comparisons

Correlation analyses

Pearson correlation analysis revealed that urinary GPC5 was strongly positively correlated with HbA1c ($r = 0.68$, $p < 0.001$; Figure 2). This indicates that poorer glycaemic control is associated with higher GPC5 excretion. The coefficient of determination ($r^2 = 0.46$) suggests that HbA1c explains approximately 46% of the variance in urinary GPC5.

Conversely, urinary GPC5 was strongly negatively correlated with eGFR ($r = –0.76$, $p < 0.001$; Figure 3). The r^2 value of 0.57 indicates that 57% of the variability in GPC5 is shared with eGFR, underscoring the close relationship between GPC5 and kidney function decline. No significant correlations were found between GPC5 and age, sex, or BMI.

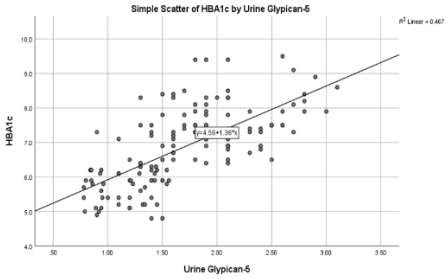


Figure 2: Scatter plot of urinary glypican-5 vs. HbA1c

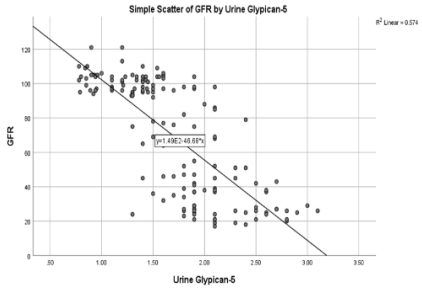


Figure 3: Scatter plot of urinary glypican-5 vs. eGFR

Discussion

This cross-sectional study investigated the association between urinary glypican-5 concentration and diabetic nephropathy in a cohort of Iraqi patients with type 2 diabetes. Our main findings are: (i) urinary GPC5 is significantly elevated in diabetic patients compared to healthy controls; (ii) GPC5 levels increase progressively with worsening albuminuria and declining eGFR; (iii) importantly, GPC5 is already elevated in normoalbuminuric patients and in those with normal eGFR; and (iv) GPC5 correlates strongly with HbA1c and eGFR. These results support the hypothesis that urinary GPC5 may serve as an early biomarker for diabetic nephropathy, potentially detecting disease before conventional markers become abnormal.

Comparison with previous studies

Our findings are consistent with the seminal work of Okamoto et al., who first identified GPC5 as a podocyte-associated heparan sulfate proteoglycan upregulated in diabetic kidney disease (12, 13). They demonstrated that urinary GPC5 originates from podocytes and that its expression increases in glomerular mesenchymal cells under hyperglycaemic conditions. Later, Ruizhao Li and colleagues showed that urinary GPC5 levels were significantly higher in type 2 diabetic patients with nephropathy than in those without nephropathy, and that GPC5 correlated positively with albuminuria and negatively with eGFR (14). Our results replicate these associations in a Middle Eastern population, extending the geographical validity of GPC5 as a candidate biomarker.

More recently, El-Kafrawy et al. reported that urinary GPC5 concentrations were significantly higher in diabetic patients with renal involvement compared to healthy controls and that levels increased with DN stage (16). Our data confirm this trend, and we further demonstrate that the

elevation is detectable even in the normoalbuminuric stage, a finding with important clinical implications. Current guidelines recommend annual UACR screening for early DN, but up to 50% of patients with declining eGFR do not have concomitant albuminuria (4, 17). Urinary GPC5 could potentially fill this diagnostic gap.

Biological plausibility

The biological rationale for urinary GPC5 as a marker of early DN is strong. Under physiological conditions, heparan sulfate proteoglycans, including GPC5, contribute to the negative charge of the glomerular basement membrane (GBM), which restricts albumin filtration. Chronic hyperglycaemia leads to non-enzymatic glycation of GBM proteins, reduced heparan sulfate expression, and loss of charge selectivity (8). Paradoxically, GPC5 itself is upregulated in diabetic glomeruli, as shown by Okamoto et al. (13). This upregulation appears to be mediated by FGF2 signalling: hyperglycaemia stimulates mesangial cells to produce GPC5, which then binds and concentrates FGF2, creating a positive feedback loop that drives mesangial matrix expansion, podocyte foot process effacement, and ultimately proteinuria (18, 19). The shedding of GPC5 into urine may represent a compensatory mechanism or simply a marker of podocyte injury. Our correlation data (GPC5 positively correlated with HbA1c) support the role of chronic hyperglycaemia in driving GPC5 excretion.

Clinical implications

The ability of urinary GPC5 to detect early kidney damage in normoalbuminuric patients is arguably the most clinically relevant finding of this study. Current ADA and KDIGO guidelines recommend initiation of reno-protective therapies (ACE inhibitors, ARBs, SGLT2 inhibitors) upon detection of microalbuminuria. However, by the time microalbuminuria appears, significant irreversible glomerular and tubulointerstitial fibrosis may have already occurred (2, 20, 21). A biomarker that identifies patients at risk before microalbuminuria could enable earlier intervention, potentially slowing or preventing progression to end-stage kidney disease. Urinary GPC5, if validated in prospective studies, could be used to stratify normoalbuminuric diabetic patients into low- and high-risk categories, guiding more intensive glucose and blood pressure control as well as earlier use of SGLT2 inhibitors or finerenone.

Nevertheless, several hurdles remain before clinical translation. The optimal cut-off value for urinary GPC5 has not been established, and the assay requires standardisation across laboratories. Our study did not perform a receiver operating characteristic (ROC) analysis to determine sensitivity and specificity, which is a limitation. Moreover, the incremental value of GPC5 over and above UACR and eGFR needs to be tested in head-to-head comparisons.

Study limitations

This study has several limitations that must be acknowledged.

1. Cross-sectional design: The most important limitation is the cross-sectional nature of the study. While we observed strong associations, we cannot establish causality or the temporal sequence between rising GPC5 and declining kidney function. A longitudinal study with repeated measurements is needed to determine whether an increase in urinary GPC5 precedes the development of microalbuminuria or eGFR decline.
2. Single-centre, modest sample size: The study was conducted in a single hospital in Kirkuk, Iraq, with 150 participants. The sample size, particularly within the normoalbuminuric subgroup (n=27), limits statistical power for some comparisons (e.g., the non-significant difference between normoalbuminuria and microalbuminuria may be a type II error). External validation in larger, multi-ethnic cohorts is required.
3. Lack of ROC analysis: We did not calculate the area under the ROC curve (AUC), sensitivity, specificity, or optimal cut-off values for GPC5. Therefore, we cannot quantify its diagnostic performance. Future studies should include ROC analysis to compare GPC5 with UACR and eGFR.
4. No adjustment for key confounders: Although we measured age, sex, and BMI, we did not collect data on blood pressure, use of renin-angiotensin system blockers, SGLT2 inhibitors, or GLP-1 receptor agonists, all of which can affect albuminuria and possibly GPC5 levels. The higher age of female patients and the unexpectedly lower BMI in the diabetic group (compared to controls) suggest possible selection bias or residual confounding.
5. Single urinary measurement: UACR is ideally measured on three separate occasions over three months to confirm persistent albuminuria. We used a single spot urine sample due to the cross-sectional design, which may have misclassified some patients (e.g., transient albuminuria due to infection or exercise). Similarly, GPC5 levels may vary diurnally; we did not assess this.
6. Lack of mechanistic data: While we discuss potential mechanisms involving FGF2 signalling, our study provides no direct experimental evidence. The discussion of pathophysiology is

largely speculative and derived from animal studies.
7. Assay standardisation: The ELISA kit used is a research-grade assay, not a clinical diagnostic kit. Inter-laboratory variability remains an issue.

Future directions
Prospective cohort studies are urgently needed to: (i) establish reference ranges and cut-off values for urinary GPC5 in different populations; (ii) determine whether baseline GPC5 predicts incident microalbuminuria or rapid eGFR decline; (iii) compare the predictive performance of GPC5 with emerging biomarkers such as urinary nephrin, KIM-1, and NGAL; and (iv) investigate whether GPC5 levels respond to reno-protective treatments (e.g., SGLT2 inhibitors). Additionally, experimental studies using podocyte-specific GPC5 knockout mice could clarify whether GPC5 is merely a marker or a pathogenic mediator of DN.

Conclusion
This study demonstrates that urinary glypican-5 concentration is significantly elevated in patients with type 2 diabetes compared to healthy controls, and that levels increase progressively with worsening albuminuria and declining estimated glomerular filtration rate. Notably, urinary GPC5 is already raised in normoalbuminuric patients and in those with normal eGFR, suggesting that it may detect early diabetic kidney injury before conventional markers become abnormal. Urinary GPC5 correlates strongly with glycaemic control (HbA1c) and inversely with kidney function (eGFR). While these findings support the potential of urinary GPC5 as an early diagnostic biomarker for diabetic nephropathy, the cross-sectional design and modest sample size preclude definitive conclusions. Prospective validation studies with ROC analysis and adjustment for confounders are required before clinical application.

List of Abbreviations
ACE: Angiotensin converting enzyme
ADA: American Diabetes Association
ANOVA: Analysis of variance
ARB: Angiotensin II receptor blocker
BMI: Body mass index
CKD EPI: Chronic Kidney Disease Epidemiology Collaboration
DN: Diabetic nephropathy
eGFR: Estimated glomerular filtration rate
ELISA: Enzyme-linked immunosorbent assay
FBG: Fasting blood glucose
FGF2: Fibroblast growth factor 2
GBM: Glomerular basement membrane
GLP 1: Glucagon-like peptide 1
GPC5: Glypican 5
HbA1c: Glycated haemoglobin
HSD: Honest significant difference
KDIGO: Kidney Disease: Improving Global Outcomes
KIM 1: Kidney injury molecule 1
NGAL: Neutrophil gelatinase-associated lipocalin
ROC: Receiver operating characteristic
SD: Standard deviation
SGLT2: Sodium glucose cotransporter 2
SPSS: Statistical Package for the Social Sciences
T2DM: Type 2 diabetes mellitus
UACR: Urine albumin to creatinine ratio

Declarations
Ethics Approval and Consent to Participate
This study was conducted in accordance with the Declaration of Helsinki (1964, revised 2013). Ethical approval was obtained from the Scientific Committee of the College of Medicine, University of Tikrit (approval reference: TU/COM/2024/143, dated 15 September 2024). Written informed consent was obtained from all individual participants included in the study. Participants were informed of their right to withdraw at any time without consequences.

Consent for Publication
All authors gave consent for the publication of this work under the Creative Commons Attribution Non-Commercial 4.0 International license. No person's data (e.g., images, names) is presented, so no additional consent is required.

Data Availability
The datasets generated and analysed during the current study are available from the corresponding author on reasonable request, subject to institutional data sharing policies and protection of patient confidentiality.

Competing Interests
The authors declare that they have no competing interests. No financial or non-financial relationships exist that could influence the reported work.

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Author Contributions
AWOK worked on the conceptualisation, methodology, investigation, formal analysis,

writing of the original draft, writing of the review & editing, project administration.
ZSA worked on the methodology, validation, supervision, and writing of the review & editing.
MOT worked on the investigation, data curation, visualisation, and writing of the original draft.
All authors read and approved the final manuscript.

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