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Combined assessment of serum netrin-1 and GDF-15 across albuminuria stages in type 2 diabetes

Netrin-1 and GDF-15 in Diabetic Kidney Disease

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Abstract

Objective: The urinary albumin-to-creatinine ratio (uACR) has limited sensitivity for early structural changes in diabetic kidney disease (DKD), prompting interest in novel circulating biomarkers. This cross-sectional study evaluated the combined diagnostic association of serum Netrin-1 and Growth Differentiation Factor-15 (GDF-15) across albuminuria stages in type 2 diabetes mellitus (T2DM).

Methods: Sixty T2DM patients (stratified by albuminuria: normo-, micro-, and macroalbuminuria, n=20 each) and 60 age- and BMI-matched healthy controls were enrolled. Serum Netrin-1 and GDF-15 were quantified by sandwich ELISA. Estimated glomerular filtration rate (eGFR) was calculated using the race-free CKD-EPI 2021 equation. ROC analysis and multivariable logistic regression were performed.

Results: Netrin-1 and GDF-15 were significantly elevated in T2DM versus controls (p=0.004 and p=0.001, respectively). Netrin-1 peaked in microalbuminuria (495.2±102.7 pg/mL) while GDF-15 was highest in macroalbuminuria (406.6±106.8 ng/L; ANOVA p=0.020 and p=0.041). Single-marker AUCs: Netrin-1 0.725 (95%CI:0.635-0.816), GDF-15 0.708 (95%CI:0.615-0.800); combined model AUC 0.743 (95%CI:0.655-0.831; p=0.032 vs GDF-15 alone). In adjusted analysis, only eGFR (aOR=0.945 per mL/min/1.73m², p<0.001) and BMI (aOR=1.195 per kg/m², p=0.023) independently predicted T2DM status; biomarker associations attenuated to non-significance.

Conclusion: Serum Netrin-1 and GDF-15 show stage-specific patterns across albuminuria in T2DM but provide only modest incremental diagnostic value over conventional renal indices. These findings are hypothesis-generating; longitudinal studies with hard renal endpoints are required to establish clinical utility.

Keywords: Netrin-1, Growth Differentiation Factor-15, Diabetic Kidney Disease, Type 2 Diabetes Mellitus, Albuminuria

Plain English Summary

This study investigated whether two substances in the blood, called Netrin-1 and GDF-15, could help detect kidney damage in people with type 2 diabetes. Current tests for diabetic kidney disease rely on measuring protein in urine. However, these tests may not detect very early kidney damage. New, simpler blood tests could potentially help doctors identify kidney problems sooner.

We measured Netrin-1 and GDF-15 in blood samples from 60 people with type 2 diabetes and 60 healthy people of similar age and weight. The patients with diabetes were divided into three groups based on the amount of protein in their urine (normal, moderate, and high).

Both substances were higher in people with diabetes compared to healthy people. Netrin-1 was highest in patients with moderate protein in their urine, while GDF-15 was highest in those with high protein levels. When the two tests were combined, they were slightly better at identifying diabetes than using either test alone. However, when the researchers accounted for kidney function and weight, the blood tests were no longer strong predictors of diabetes.

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These blood tests show different patterns depending on the stage of kidney damage in diabetes. However, they are not yet reliable enough to replace existing tests. More research, especially studies that follow patients over time, is needed to determine whether these tests could be useful in routine medical practice.

Introduction

Diabetes mellitus has emerged as one of the most pervasive global health challenges of the twenty-first century, representing a leading cause of morbidity and premature mortality worldwide. Epidemiological projections indicate that the prevalence of diabetes is accelerating at an alarming rate, with estimates suggesting that over 780 million individuals will be affected by 2045 (1). Type 2 diabetes mellitus (T2DM) constitutes most of these cases, accounting for approximately 90% of all diabetes diagnoses (2). The pathophysiology of T2DM is primarily characterised by a dual mechanism: peripheral insulin resistance and progressive pancreatic β-cell dysfunction, which collectively result in chronic hyperglycaemia (3).

Among the microvascular complications associated with T2DM, diabetic kidney disease (DKD) stands out as the leading cause of end-stage renal disease globally (4). The structural hallmarks of DKD include glomerular basement membrane thickening, mesangial matrix expansion, podocyte loss, and nodular glomerulosclerosis (5). The pathogenesis of these structural alterations is multifactorial, driven by chronic hyperglycaemia, which promotes the accumulation of advanced glycation end products (AGEs), dysregulates growth factor signalling, and activates protein kinase C pathways. This metabolic disruption subsequently exacerbates oxidative stress and chronic inflammation, leading to early haemodynamic changes including glomerular hyperfiltration and elevated intraglomerular pressure (6). Ultimately, these synergistic mechanisms induce cellular apoptosis and extracellular matrix remodelling, clinically manifesting as progressive albuminuria and systemic hypertension (7).

Albuminuria has long served as the cornerstone clinical indicator for the detection and monitoring of DKD. Its presence reflects compromised integrity of the glomerular filtration barrier. Clinically, urinary albumin excretion is stratified into microalbuminuria (urinary albumin-to-creatinine ratio [uACR] 30–300 mg/g) and macroalbuminuria (uACR >300 mg/g), both of which strongly correlate with progressive renal decline and cardiovascular events (8). Unlike serum creatinine, which only rises after substantial loss of renal function, microalbuminuria provides a critical window for detecting early-stage renal impairment before a decline in estimated glomerular filtration rate (eGFR) (9). However, the diagnostic utility of uACR is limited by its inability to detect the earliest structural renal changes that occur during the normoalbuminuric phase of DKD, sometimes termed the "silent" phase. This limitation has motivated the search for more sensitive biomarkers that could identify patients at risk before the onset of clinically detectable albuminuria.

Netrin-1 has emerged as a promising biomarker for early renal injury. Originally identified as a neural guidance cue, Netrin-1 is a signalling protein that coordinates cellular migration and modulates immune cell chemotaxis (10). Its biological activity is primarily mediated through interaction with the Uncoordinated-5-B receptor, triggering anti-inflammatory cascades including suppression of NF-κB signalling and attenuation of pro-inflammatory cytokine production (11). In response to renal injury, Netrin-1 expression is rapidly upregulated in proximal tubular epithelial cells (12). Emerging evidence suggests that urinary and serum Netrin-1 levels are elevated in diabetic patients and may serve as an early indicator of tubulointerstitial damage (13). The rapid upregulation of Netrin-1 in response to tubular stress makes it a biologically plausible marker for detecting renal injury at its earliest stages, potentially before the development of microalbuminuria.

Concurrently, Growth Differentiation Factor-15 (GDF-15), a stress-responsive member of the transforming growth factor-β superfamily, has garnered attention in the context of metabolic and renal diseases (14, 15). Under physiological conditions, GDF-15 expression is minimal, but it is robustly induced in response to cellular stress, inflammation, and tissue injury (15). GDF-15 may exert renoprotective effects by inhibiting NF-κB and JNK pathways, thereby mitigating inflammation and apoptosis in endothelial cells and podocytes (16). Furthermore, GDF-15 has been shown to suppress the AGE/RAGE signalling axis (17). Consequently, elevated circulating GDF-15 levels are recognised as a potent biomarker for the presence and progression of DKD (18). Unlike Netrin-1, which appears to be more specifically linked to early tubular injury, GDF-15 reflects a broader stress response that may become more prominent as kidney disease advances.

Despite the individual diagnostic potential of Netrin-1 and GDF-15, evidence regarding their combined performance across the progressive stages of albuminuria in T2DM remains limited.

The distinct biological roles of these markers, Netrin-1 as an indicator of early tubular injury and GDF-15 as a marker of progressive cellular stress, suggest that they may offer complementary information when assessed together across the albuminuria spectrum. However, the extent to which combined assessment improves diagnostic discrimination over either marker alone has not been adequately explored in Iraqi populations.

Therefore, this cross-sectional study aimed to: (1) characterise serum Netrin-1 and GDF-15 concentrations across albuminuria stages in T2DM patients compared with healthy controls; (2) evaluate their unadjusted and adjusted associations with T2DM status; and (3) assess whether their combined assessment improves discrimination over either marker alone. It is explicitly acknowledged that prediction of future renal events cannot be inferred from the present cross-sectional design, and all findings should be interpreted as hypothesis-generating rather than clinically definitive.

Materials and Methods

Study Design and Population

A cross-sectional, single-centre, hospital-based case–control study was conducted at Merjan Teaching Hospital, Hilla, Iraq, from November 2025 to February 2026. Participants were enrolled by consecutive sampling from the diabetes outpatient clinic during the study period. Healthy volunteers were recruited from staff and accompanying relatives over the same period. This approach was pragmatic given resource constraints but introduces potential selection bias, which is acknowledged as a limitation.

The study population comprised 120 participants: 60 patients with T2DM (mean age 60.5 ± 8.19 years; range 41–75) and 60 apparently healthy volunteers matched for age and body mass index (BMI; mean age 61.1 ± 6.59 years; range 51–72). Sex was not used as an explicit matching criterion; however, the male-to-female ratio between the two groups did not differ significantly (37/23 vs 32/28; χ^2 p=0.449), and sex was therefore included as a covariate in adjusted models.

To evaluate the progression of DKD, the T2DM cohort was further stratified into three subgroups (n=20 each) based on uACR, in accordance with KDIGO 2024 guidelines: normoalbuminuria (uACR <30 mg/g), microalbuminuria (uACR 30–300 mg/g), and macroalbuminuria (uACR >300 mg/g).

Sample Size Considerations

The achieved sample size of 60 patients and 60 controls provides approximately 88% power at a two-sided α of 0.05 to detect a between-group standardised mean difference of d ≈ 0.55 in serum biomarker concentrations, equivalent to the effect sizes reported for circulating Netrin-1 and GDF-15 in prior DKD cohorts (14, 19). Within the three albuminuria subgroups (n=20 each), the design provides ≥80% power to detect a one-way ANOVA effect size of f ≈ 0.45 across the three strata. We acknowledge that this is a post-hoc justification rather than a prospectively derived target, and the sample size therefore primarily supports description and unadjusted comparisons. Multivariable analyses with multiple covariates are interpreted accordingly, recognising the constraints imposed by the modest sample size.

Inclusion and Exclusion Criteria

The primary inclusion criterion for the patient cohort was a confirmed diagnosis of T2DM in accordance with the World Health Organisation diagnostic criteria, encompassing individuals aged 41–75 years. Exclusion criteria for both patients and controls included: type 1 diabetes mellitus, any malignancy, acute or chronic inflammatory or infectious diseases, hepatic dysfunction, established renal failure unrelated to DKD, pregnancy, active smoking, and chronic alcoholism. These exclusion criteria were applied to minimise confounding from conditions that could independently affect circulating biomarker levels.

Sample Collection and Processing

Venous blood samples (approximately 5 mL) and random midstream spot urine samples were collected from all participants under standardised fasting conditions (≥8 hours fasting). Blood was allowed to clot at room temperature for 30 minutes before centrifugation at 3000 rpm for 15 minutes to separate the serum. Aliquoted serum was stored at −80°C until analysis. All samples were thawed immediately before analysis to minimise pre-analytical variability. This single freeze-thaw cycle is consistent with manufacturer recommendations and minimises protein degradation.

Routine Biochemical Assays and eGFR Calculation

General biochemical parameters were measured on a fully automated Dirui CS-T180 chemistry analyser (Dirui Industrial Co., Ltd., Changchun, China). Parameters included fasting blood sugar (FBS), serum creatinine, and urinary microalbumin. Glycated haemoglobin (HbA1c) was quantified by HPLC on a GH-900 Plus Lifotronic analyser (Lifotronic Technology Co., Ltd., Shenzhen, China).

The urinary albumin-to-creatinine ratio (uACR) was calculated as follows: uACR (mg/g) = [Urine albumin (mg/L) ÷ Urine creatinine (g/L)] × 100.
Estimated glomerular filtration rate (eGFR) was calculated for each participant using the race-free CKD-EPI 2021 creatinine equation: eGFR = 142 × min(Scr/κ, 1)^α × max(Scr/κ, 1)^−1.200 × 0.9938^Age × 1.012 [if female], where κ = 0.7 (female) or 0.9 (male) and α = −0.241 (female) or −0.302 (male). The 2021 CKD-EPI equation was selected because it removes race adjustment, making it more appropriate for diverse populations and consistent with contemporary guidelines.

Quantification of Netrin-1 and GDF-15 by ELISA
Serum concentrations of Netrin-1 and GDF-15 were determined using commercially available sandwich ELISA kits (Bioassay Technology Laboratory, Shanghai, China; catalogue numbers E1277Hu for Netrin-1 and E0037Hu for GDF-15), strictly following the manufacturer's protocols. According to the manufacturer's specifications, the kits provide a sensitivity (lower limit of detection) of 5.13 pg/mL for Netrin-1 and 1.05 ng/L for GDF-15; assay measuring ranges of 10–3000 pg/mL (Netrin-1) and 2–600 ng/L (GDF-15); intra-assay coefficient of variation <8%; and inter-assay coefficient of variation <10%. All samples were assayed in duplicate, and the mean of the two readings was used for analysis. Standard curves and the manufacturer's quality-control samples were run on every plate; runs with QC values outside the manufacturer's acceptance limits or with duplicate CV >15% were repeated. Plates were read on a Tecan Sunrise microplate reader at 450 nm with reference correction at 630 nm.

Reporting Guidelines and Missing Data
This study was conducted and is reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for cross-sectional studies[38]. A completed STROBE checklist is provided as a supplementary file. Data were collected from all 120 enrolled participants; no participant had missing values for the variables analysed (age, sex, BMI, FBS, HbA1c, serum creatinine, uACR, Netrin-1, GDF-15), so analyses were performed on the complete dataset without imputation.

Statistical Analysis
All statistical analyses were performed using GraphPad Prism v10.0 (GraphPad Software, Boston, MA, USA) and IBM SPSS Statistics v26.0 (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to assess the normality of continuous variables. Normally distributed continuous variables are presented as mean ± standard deviation (SD); non-normally distributed variables are presented as median with interquartile range (IQR; Q1–Q3). Categorical variables are presented as absolute frequencies and percentages.
Differences between two independent groups were assessed using the independent-samples t-test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. Categorical variables were compared using the χ^2 (chi-square) test. Differences across the three T2DM albuminuria subgroups were assessed using one-way ANOVA with Tukey's post-hoc test for normally distributed variables, and the Kruskal–Wallis test with Dunn's post-hoc for non-normally distributed variables. Bivariate associations were examined using Spearman's rank correlation coefficient (denoted Rs) rather than Pearson's r, given that several variables (notably uACR and creatinine) showed skewed distributions. This non-parametric approach is more robust for data that do not meet normality assumptions.
For clinical interpretability of regression coefficients, biomarker variables were rescaled. Netrin-1 was divided by 100 (per 100 pg/mL increment) and GDF-15 by 50 (per 50 ng/L increment) in the logistic regression models. This rescaling makes odds ratios more clinically meaningful than per-unit values that are too small to interpret practically.
The diagnostic performance of Netrin-1 and GDF-15 was evaluated by receiver-operating-characteristic (ROC) curve analysis. Area under the curve (AUC) was reported with 95% confidence intervals. Optimal cut-offs were chosen by maximising Youden's J statistic. A combined biomarker score was constructed as the predicted probability from a multivariable logistic regression of T2DM status on Netrin-1 and GDF-15, and the AUC of this combined score was compared with each single-marker AUC using the DeLong test for correlated ROC curves. It is important to note that the ROC target was T2DM status versus controls, not DKD progression or renal outcomes, and this limitation is reflected in the interpretation.
To assess independent associations of biomarkers with T2DM status while controlling for measured confounders, multivariable binary logistic regression was performed with T2DM (yes/no) as the dependent variable and rescaled Netrin-1, rescaled GDF-15, age, sex, BMI, and eGFR as predictors. Model fit was assessed using the Hosmer–Lemeshow goodness-of-fit test, with predictive accuracy quantified by McFadden pseudo-R^2. Multicollinearity was screened by variance inflation factor (VIF), with VIF<5 considered acceptable. Adjusted odds

ratios (aORs) are reported per 100 pg/mL increment for Netrin-1 and per 50 ng/L increment for GDF-15, with 95% confidence intervals (Wald). A two-sided p-value <0.05 was considered statistically significant.

Results

Baseline Participant Characteristics

Table 1 presents the demographic and clinical characteristics of the study population. There were no significant differences between the T2DM and control groups in age (60.5 ± 8.19 vs 61.1 ± 6.59 years, p=0.720), sex distribution (37/23 vs 32/28; p=0.449), or BMI (29.2 ± 5.9 vs 26.7 ± 1.36 kg/m², p=0.120). The lack of significant difference in BMI is noteworthy, as obesity is a confounder that could influence biomarker levels independent of diabetes status. As expected, T2DM patients exhibited markedly poorer glycaemic control. Mean FBS was 204.0 ± 50.0 mg/dL in patients versus 103.6 ± 10.8 mg/dL in controls (p<0.001), and HbA1c was 8.60 ± 1.70% versus 5.62 ± 0.67% (p<0.001). These values indicate that the patient cohort had, on average, suboptimal glycaemic control, reflecting the challenges of diabetes management in this setting.

Serum creatinine was significantly higher in T2DM patients (1.7 ± 1.0 vs 0.85 ± 0.25 mg/dL, p<0.001), and median uACR was substantially elevated (862, IQR 29.4–769 vs 22.3, IQR 10.7–26.6 mg/g, p<0.001 by Mann–Whitney U). Mean eGFR (CKD-EPI 2021) was 88.0 ± 19.2 mL/min/1.73 m² in controls and 48.2 ± 29.1 mL/min/1.73 m² in T2DM patients (mean difference −35.7 mL/min/1.73 m², 95% CI −45.0 to −26.4; p<0.001). The substantial reduction in eGFR among patients reflects the advanced renal impairment present in this cohort.

Serum Netrin-1 (497.8 ± 148.2 vs 411.5 ± 76.6 pg/mL, p=0.004) and GDF-15 (379.1 ± 136.1 vs 304.1 ± 78.4 ng/L, p=0.001) were both significantly elevated in T2DM patients compared with controls. These elevations are consistent with the hypothesis that both markers reflect renal stress and injury associated with diabetes.

Table 1: Demographic, clinical, and biomarker characteristics of T2DM patients and controls

Variable	Controls (n=60)	T2DM patients (n=60)	p-value	Test
Demographic characteristics				
Age (years)	61.1 ± 6.59	60.5 ± 8.19	0.72	t-test
Male / Female, n (%)	32 (53.3) / 28 (46.7)	37 (61.7) / 23 (38.3)	0.449	χ^2
BMI (kg/m²)	26.7 ± 1.36	29.2 ± 5.9	0.12	MW-U
Albuminuria status (T2DM only)				
Normoalbuminuria, n (%)	---	20 (33.3)	---	---
Microalbuminuria, n (%)	---	20 (33.3)	---	---
Macroalbuminuria, n (%)	---	20 (33.3)	---	---
Metabolic and biochemical findings				
FBS (mg/dL)	103.6 ± 10.8	204.0 ± 50.0	<0.001	t-test
HbA1c (%)	5.62 ± 0.67	8.60 ± 1.70	<0.001	MW-U
Serum creatinine (mg/dL)	0.85 ± 0.25	1.7 ± 1.0	<0.001	MW-U
eGFR (mL/min/1.73 m², CKD-EPI 2021)	88.0 ± 19.2	48.2 ± 29.1	<0.001	MW-U
uACR (mg/g), median (IQR)	22.3 (10.7–26.6)	862 (29.4–769)	<0.001	MW-U
Novel biomarkers				
Netrin-1 (pg/mL)	411.5 ± 76.6	497.8 ± 148.2	0.004	t-test
GDF-15 (ng/L)	304.1 ± 78.4	379.1 ± 136.1	0.001	t-test

Data are mean ± SD or median (IQR) for continuous variables and n (%) for categorical variables. Units standardised to pg/mL for Netrin-1 and ng/L for GDF-15 throughout. eGFR added (CKD-EPI 2021). t-test = independent-samples t-test; MW-U = Mann–Whitney U test; χ^2 = chi-square test

Biomarker Distribution Across Albuminuria Stages

Across the three T2DM subgroups, Netrin-1 levels showed significant variation (ANOVA p=0.020), peaking in the microalbuminuria subgroup (495.2 ± 102.7 pg/mL) and remaining significantly elevated relative to normoalbuminuria (449.1 ± 68.5 pg/mL). The peak at the microalbuminuria stage is consistent with Netrin-1's proposed role as an early marker of tubular injury that may precede the development of more advanced renal damage.

GDF-15 levels also varied significantly across the subgroups (p=0.041), characterised by a distinct elevation in the macroalbuminuria subgroup (406.6 ± 106.8 ng/L) compared with normoalbuminuria (358.8 ± 75.1 ng/L) and microalbuminuria (348.1 ± 72.7 ng/L). The progressive increase in GDF-15 with advancing albuminuria suggests that this marker reflects cumulative cellular stress and injury that becomes more prominent as kidney disease advances.

Table 2 summarises these findings, and Figures 1 and 2 provide visual representations of the biomarker distributions across the study groups.

Table 2: Serum Netrin-1 and GDF-15 across albuminuria stages in T2DM patients

Biomarker	Normoalbuminuria (n=20)	Microalbuminuria (n=20)	Macroalbuminuria (n=20)	p (ANOVA)
Netrin-1 (pg/mL)	449.1 ± 68.5 ^{A}	495.2 ± 102.7 ^{B}	477.7 ± 60.7 ^{B}	0.02
GDF-15 (ng/L)	358.8 ± 75.1 ^{A}	348.1 ± 72.7 ^{A}	406.6 ± 106.8 ^{B}	0.041

Data are mean ± SD. Different superscript letters (A, B) within a row denote significant differences between the indicated groups based on Tukey's post-hoc multiple-comparisons test (p<0.05); identical letters indicate no significant difference

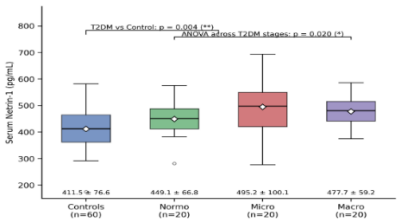

Figure 1: Boxplot of serum Netrin-1 (pg/mL) across study groups

Box = IQR with median line; diamond = mean; whiskers = 1.5×IQR. Mean ± SD shown below each box

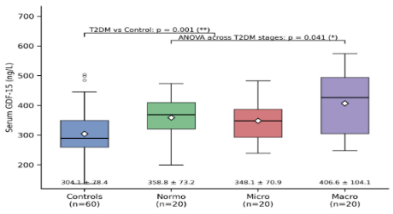

Figure 2: Boxplot of serum GDF-15 (ng/L) across study groups

Box = IQR with median line; diamond = mean; whiskers = 1.5×IQR. Mean ± SD shown below each box

Bivariate Correlations

Spearman's rank correlation analysis (Rs) within the T2DM cohort (n=60) showed a significant positive correlation between Netrin-1 and GDF-15 (Rs=0.380, p=0.003). This correlation, while statistically significant, is moderate in magnitude, suggesting that these markers capture overlapping but distinct aspects of renal pathology.

Netrin-1 was positively correlated with FBS (Rs=0.287, p=0.026) and HbA1c (Rs=0.418, p=0.001), indicating that poorer glycaemic control is associated with higher Netrin-1 levels. This is consistent with the hypothesis that hyperglycaemia drives tubular injury reflected by Netrin-1 elevation. Netrin-1 was weakly correlated with uACR (Rs=0.168, p=0.20) and inversely with eGFR (Rs=−0.240, p=0.066). The lack of a strong correlation with uACR suggests that Netrin-1 captures aspects of renal injury not fully reflected by albuminuria.

GDF-15 correlated positively with uACR (Rs=0.287, p=0.026), supporting its association with more advanced renal involvement. The correlation with eGFR was not statistically significant (Rs=0.234, p=0.072). These correlation patterns support the stage-specific profiles observed in the subgroup analysis.

Table 3 presents the complete correlation matrix, and Figure 3 provides a scatter plot illustrating the relationship between the two biomarkers.

Table 3: Spearman's rank correlations (Rs) between biomarkers and clinical/glycaemic indices in T2DM patients (n=60)

Variable	Netrin-1 (Rs, p)	GDF-15 (Rs, p)	uACR / eGFR (Rs, p)
Netrin-1	---	Rs=0.380, p=0.003	uACR Rs=0.168, p=0.20; eGFR Rs=−0.240, p=0.066
GDF-15	Rs=0.380, p=0.003	---	uACR Rs=0.287, p=0.026; eGFR Rs=0.234, p=0.072
FBS	Rs=0.287, p=0.026	Rs=0.113, p=0.39	---
HbA1c	Rs=0.418, p=0.001	Rs=0.002, p=0.99	---

Rs = Spearman's rank correlation coefficient

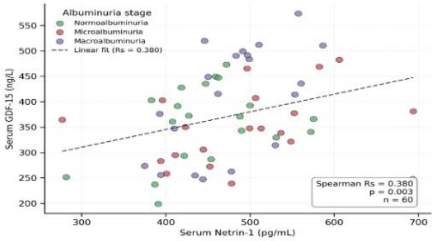

Figure 3: Scatter plot of serum Netrin-1 versus serum GDF-15 in T2DM patients (n=60)

Spearman Rs=0.380, p=0.003. Points coloured by albuminuria stage

Diagnostic Performance and Combined Biomarker Analysis

ROC curve analysis was used to evaluate the discriminative performance of Netrin-1 and GDF-15 for distinguishing T2DM patients from healthy controls. It is important to emphasise that the diagnostic target was T2DM status, not DKD progression, and the modest AUC values should be interpreted accordingly.

Netrin-1 yielded an AUC of 0.725 (95% CI 0.635–0.816, p<0.001); at an optimal cut-off of >434.6 pg/mL, sensitivity was 73.6%, specificity 63.3%, PPV 79.2%, and NPV 55.9%. GDF-15 yielded an AUC of 0.708 (95% CI 0.615–0.800, p<0.001); at >295.0 ng/L, sensitivity was 79.8%, specificity 50.0%, PPV 76.9%, and NPV 61.2%. The higher sensitivity but lower specificity of GDF-15 at its optimal cut-off suggests that it may be more useful for ruling out disease than for confirming it.

A logistic-regression-derived combined score was constructed (logit = β_0 + β_1·Netrin-1 + β_2·GDF-15) and its ROC was compared with each single-marker ROC using the DeLong test (Figure 4). The combined model achieved an AUC of 0.743 (95% CI 0.655–0.831), representing a modest, statistically non-significant improvement over Netrin-1 alone (ΔAUC=0.018; DeLong p=0.65) but a significant improvement over GDF-15 alone (ΔAUC=0.035; DeLong p=0.032). At the Youden-optimal cut-off, the combined model achieved sensitivity 71.7%, specificity 71.7%, PPV 71.2%, and NPV 70.5%.

The finding that the combined model does not significantly outperform Netrin-1 alone is important. It suggests that the additional information provided by GDF-15 may be limited once Netrin-1 is already measured. This has implications for the potential clinical utility of a combined biomarker panel.

Table 4 summarises the diagnostic performance characteristics, and Figure 4 presents the ROC curves.

Table 4: Diagnostic performance characteristics of Netrin-1, GDF-15, and the combined biomarker model based on ROC curve analysis

Biomarker	Cut-off	Sens (%)	Spec (%)	AUC (95% CI)	PPV / NPV (%)
Netrin-1	>434.6 pg/mL	73.6	63.3	0.725 (0.635–0.816); p<0.001	79.2 / 55.9
GDF-15	>295.0 ng/L	79.8	50	0.708 (0.615–0.800); p<0.001	76.9 / 61.2
Combined (Netrin-1 + GDF-15)	Youden's J (predicted prob.)	71.7	71.7	0.743 (0.655–0.831); p<0.001	71.2 / 70.5

*AUC = area under the ROC curve; PPV = positive predictive value; NPV = negative predictive value. DeLong test for AUC comparison: Combined vs Netrin-1 alone, ΔAUC=0.018, p=0.65; Combined vs GDF-15 alone, ΔAUC=0.035, p=0.032. Note that PPV and NPV are sample-specific and prevalence-dependent; they should not be generalised to other populations without validation

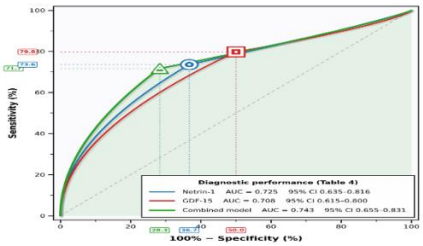

Figure 4: ROC curves for Netrin-1 (blue), GDF-15 (orange), and the combined logistic-regression-derived model (green) for distinguishing T2DM patients from healthy controls (n=120)

Markers indicate the optimal Youden cut-off for each curve: Netrin-1 (●) >434.6 pg/mL — Sens 73.6%, Spec 63.3%; GDF-15 (■) >295.0 ng/L — Sens 79.8%, Spec 50.0%; Combined (▲) — Sens 71.7%, Spec 71.7%. The diagonal grey dashed line represents the no-discrimination reference (AUC = 0.500). DeLong test for AUC comparison: Combined vs Netrin-1 alone, ΔAUC = 0.018, p = 0.65; Combined vs GDF-15 alone, ΔAUC = 0.035, p = 0.032

Univariable and Multivariable Logistic Regression

Univariable binary logistic regression confirmed that both Netrin-1 and GDF-15 were significantly associated with T2DM status in unadjusted analyses (Table 5). For Netrin-1, the odds ratio per 100 pg/mL increment was 2.96 (95% CI 1.57–6.23, p=0.001), indicating that each 100 pg/mL increase in Netrin-1 was associated with nearly a threefold increase in the odds of having T2DM. For GDF-15, the odds ratio per 50 ng/L increment was 1.66 (95% CI 1.24–2.28, p=0.001). These unadjusted associations are consistent with the biomarker elevations observed in the patient cohort.

To address the possibility of confounding and to assess the independent contribution of the biomarkers, a multivariable logistic regression was performed, including age, sex, BMI, eGFR, rescaled Netrin-1, and rescaled GDF-15 as predictors of T2DM status (Table 6, Figure 5). After adjustment, eGFR (adjusted OR=0.945 per mL/min/1.73 m², 95% CI 0.924–0.966, p<0.001) and BMI (adjusted OR=1.195 per kg/m², 95% CI 1.025–1.395, p=0.023) emerged as the only independent predictors of T2DM status. The biomarker odds ratios attenuated to non-significance (adjusted Netrin-1 OR per 100 pg/mL = 1.36, 95% CI 0.64–2.85, p=0.406; adjusted GDF-15 OR per 50 ng/L = 1.27, 95% CI 0.91–1.78, p=0.168). This attenuation suggests that part of the unadjusted association of Netrin-1 and GDF-15 with T2DM is shared with or mediated by renal function, as captured by eGFR.

The model showed good fit (Hosmer–Lemeshow χ^2=5.79, p=0.671), substantial explanatory power (McFadden pseudo-R^2=0.453), and no problematic multicollinearity (all VIF<1.4). The observation that eGFR is on the causal pathway between DKD and biomarker elevation is important; including it in the model may represent overadjustment, and the attenuation of biomarker ORs may reflect this. However, the model as specified provides a conservative estimate of the independent association of the biomarkers after accounting for renal function.

Table 5: Univariable binary logistic regression for T2DM status

Variable	B	SE	Odds Ratio	95% CI	p-value
Netrin-1 (per 100 pg/mL)	1.087	0.342	2.96	1.57–6.23	0.001
GDF-15 (per 50 ng/L)	0.508	0.155	1.66	1.24–2.28	0.001

Each row represents a separate univariable model

Table 6: Multivariable adjusted logistic regression for T2DM status (n=120; adjusted for age, sex, BMI, eGFR, Netrin-1, GDF-15)

Variable	B	SE	Adjusted OR	95% CI	p-value
Age (per year)	−0.072	0.04	0.931	0.860–1.008	0.076
Male sex (vs female)	0.857	0.614	2.357	0.707–7.860	0.163
BMI (per kg/m²)	0.179	0.079	1.195	1.025–1.395	0.023*
eGFR (per mL/min/1.73 m²)	−0.057	0.011	0.945	0.924–0.966	<0.001*
Netrin-1 (per 100 pg/mL)	0.307	0.376	1.36	0.64–2.85	0.406
GDF-15 (per 50 ng/L)	0.239	0.172	1.27	0.91–1.78	0.168

Hosmer–Lemeshow χ^2=5.79, p=0.671 (good fit). McFadden pseudo-R^2=0.453. All VIF<1.4 (no multicollinearity). * p<0.05

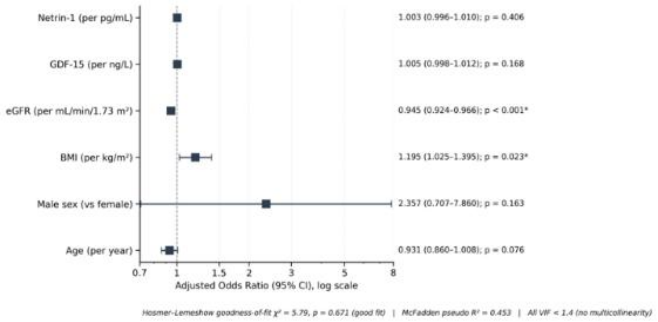

Figure 5: Forest plot of adjusted odds ratios from the multivariable logistic regression model
eGFR and BMI were the only independent predictors of T2DM status; the biomarker associations attenuated to non-significance after adjustment

Discussion

The present cross-sectional study evaluated the diagnostic association of serum Netrin-1 and GDF-15 with T2DM and across albuminuria stages in 60 patients with T2DM and 60 matched controls. We observed that both biomarkers were significantly elevated in T2DM compared with controls, with Netrin-1 peaking at the microalbuminuria stage and GDF-15 at the macroalbuminuria stage. These patterns are consistent with their proposed roles in early tubular injury and progressive cellular stress, respectively. However, in adjusted multivariable analysis, eGFR and BMI emerged as the only independent predictors of T2DM status, and the biomarker associations attenuated to non-significance. This attenuation indicates that an important fraction of the unadjusted association of Netrin-1 and GDF-15 with T2DM is shared with renal function, a nuance that tempers any interpretation of these markers as independent diagnostic tools.

The finding that Netrin-1 peaks at the microalbuminuria stage aligns with a recent systematic review and meta-analysis by Behnoush et al. (2024), which included 19 studies and 2,061 cases and demonstrated that diabetic patients with microalbuminuria and macroalbuminuria had significantly higher circulatory Netrin-1 levels compared to normoalbuminuric groups (19). Similarly, Ay et al. reported elevated serum Netrin-1 in patients with diabetes whose mean HbA1c was 8.1% compared to non-diabetic patients (20). The Spearman correlations we observed between Netrin-1 and FBS (Rs=0.287) and HbA1c (Rs=0.418) align with multiple reports of glycaemic association in adult cohorts (21, 22, 23).

The biological rationale for Netrin-1's elevation at the early microalbuminuric phase is well-established. Netrin-1 is rapidly upregulated in proximal tubular epithelial cells in response to renal stress and exerts anti-inflammatory effects through suppression of COX-2 and NF-κB signalling (10, 11, 12). This makes it a plausible marker for subclinical tubulointerstitial injury that may precede glomerular damage (24, 25). Our data are compatible with this paradigm, but do not establish temporal causality given the cross-sectional design.

The elevation of GDF-15 at the macroalbuminuria stage is supported by prior data showing that circulating GDF-15 rises with more advanced albuminuria and CKD progression (16, 18, 26, 27). Carlsson et al. demonstrated in a proteomics approach that elevated GDF-15 levels are associated with both DKD and future cardiovascular events in T2DM cohorts (14). Agarwal et al. found GDF-15 to have the highest rate of elevation in DKD compared with prediabetes and T2DM without nephropathy (28). Our findings extend these observations to an Iraqi T2DM cohort and reinforce the view that GDF-15 reflects systemic stress and progressive cellular injury rather than early structural changes. However, it is important to note that we cannot determine whether elevated GDF-15 precedes, accompanies, or follows the development of macroalbuminuria.

The combined biomarker analysis yielded an AUC of 0.743, a modest improvement over every single biomarker (Netrin-1 AUC 0.725; GDF-15 AUC 0.708). The DeLong test confirmed a statistically significant gain over GDF-15 alone ($p=0.032$) but not over Netrin-1 alone ($p=0.65$). This suggests that combined assessment improves discrimination over the weaker single marker but does not appreciably exceed the better single marker in this cohort. The AUCs for all models are in the modest range (0.70–0.75), and these values are not yet sufficient for clinical implementation as standalone diagnostic tests. An AUC of ≥0.80 is generally considered to indicate adequate discrimination for clinical use, and our models fall short of this threshold. External validation in independent cohorts and against clinically meaningful endpoints (eGFR slope, ESRD-free survival) is required before clinical translation can be considered.

The most important shift in interpretation introduced by the multivariable analysis concerns the original framing of these markers as independent predictors. When eGFR is included in the adjustment set, the biomarker odds ratios attenuate substantially and lose statistical significance. This pattern is biologically plausible: eGFR is a direct measure of renal function, and Netrin-1 and GDF-15 elevations partly reflect the same underlying renal injury. However, the possibility of overadjustment should be considered. If eGFR lies on the causal pathway between the disease process and biomarker elevation, including it in the model may inappropriately attenuate the association. This is an important consideration that we acknowledge as a limitation. The conservative interpretation is that the biomarkers are complementary indicators of renal involvement rather than independent predictors, and we have removed claims of risk stratification because the cross-sectional design and absence of prospective renal endpoints cannot support that conceptual framework.

Study limitations

This study has several important limitations that should be considered when interpreting the findings.

First, the cross-sectional design precludes causal inference and any conclusion about the temporal prediction of renal outcomes. We cannot determine whether elevated biomarkers precede or are a consequence of renal injury. Prospective longitudinal follow-up with hard renal endpoints (eGFR slope, ESRD-free survival) is required before these biomarkers can be considered for risk stratification.

Second, this is a single-centre study with consecutive sampling from one Iraqi hospital. The control group was recruited from staff and accompanying relatives, introducing potential selection bias. This convenience sampling approach means that the control group may not be fully representative of the general population, and the findings may not generalise to other ethnic groups, healthcare systems, or disease severities. The absence of a population-based control sample is a notable limitation.

Third, several clinically important variables were not collected under the original protocol and therefore could not be incorporated into adjusted

models, even though they may confound the biomarker–renal injury relationship. These include: blood pressure (systolic and diastolic), full lipid profile (total cholesterol, LDL, HDL, triglycerides), inflammatory markers (high-sensitivity CRP, IL-6), duration of diabetes, and current pharmacotherapy (ACE inhibitors/ARBs, SGLT2 inhibitors, GLP-1 agonists, statins, metformin, insulin). The absence of blood pressure and medication data is particularly important, as these are key determinants of renal function and may independently affect biomarker levels.

Fourth, biomarkers were measured at a single time point, and we therefore cannot account for biological variability or short-term fluctuation. This is particularly relevant for Netrin-1, which may be rapidly upregulated in response to acute tubular stress. Multiple measurements over time would provide a more stable estimate of an individual's biomarker status.

Fifth, the diagnostic performance metrics reported here have not been externally validated. The AUCs (0.71–0.74) are modest and are not yet sufficient to support clinical use as standalone diagnostic tests. The PPV and NPV are sample-specific and prevalence-dependent; they should not be generalised to other populations without independent validation.

Sixth, the multivariable model is constrained by the achieved sample size. With six predictors and 120 observations, the events-per-variable ratio is acceptable but not large, and the wide confidence intervals on some predictors (notably sex) reflect this. The model may be overfit, and the findings should be validated in a larger cohort.

Seventh, the use of verbal rather than written consent, while ethically justified in this setting, may be viewed differently by international readers. The justification for verbal consent is provided, but remains a potential limitation.

Eighth, the absence of data on the duration of diabetes means we cannot assess whether biomarker levels correlate with disease chronicity or with glycaemic control over time. This is an important gap that limits the interpretability of the correlations with cross-sectional glycaemic measures.

Future research directions

Future studies should address the limitations identified above. Specifically, prospective longitudinal studies with serial biomarker measurements and hard renal endpoints are needed to determine whether Netrin-1 and GDF-15 can predict renal decline independent of conventional risk factors. Such studies should collect comprehensive cardiometabolic and pharmacotherapy data to allow full confounder adjustment. External validation of the combined biomarker model in independent cohorts is essential before any clinical application can be considered. Additionally, studies investigating the pathophysiological mechanisms linking these biomarkers to renal injury would strengthen the biological plausibility of their use as diagnostic tools. Finally, evaluation of whether biomarker-guided risk classification can improve clinical decision-making beyond conventional renal indices is required to determine the value of these markers in practice.

Conclusion

This cross-sectional study provides further evidence that serum Netrin-1 and GDF-15 are elevated in T2DM and show distinct patterns across albuminuria stages, Netrin-1 peaking at the microalbuminuric phase and GDF-15 at the macroalbuminuric phase. Combining the two biomarkers improves discrimination modestly (AUC 0.743) and significantly outperforms GDF-15 alone, but does not statistically exceed Netrin-1 alone. After adjustment for age, sex, BMI, and eGFR, the independent association of these biomarkers with T2DM status attenuates, indicating that part of their unadjusted signal reflects renal function. The findings should therefore be interpreted as hypothesis-generating rather than clinically definitive.

The modest AUC values (0.71–0.74) indicate that these biomarkers, whether used singly or in combination, are not yet suitable for clinical implementation as standalone diagnostic tests for T2DM or DKD. Their primary value at present is as research tools for further investigation of the pathophysiology of diabetic kidney disease. Longitudinal studies with hard renal endpoints and external validation are required before Netrin-1 and GDF-15 can be considered for routine clinical use.

In summary, while Netrin-1 and GDF-15 show promise as complementary biomarkers reflecting different stages of renal involvement in T2DM, their incremental diagnostic value over conventional renal indices is modest in this cross-sectional cohort. The scientific community should view these findings as a foundation for further research rather than as a basis for clinical adoption.

List of Abbreviations

AGEs: Advanced Glycation End Products
ANOVA: Analysis of Variance
aOR: Adjusted Odds Ratio
AUC: Area Under the Curve
BMI: Body Mass Index
CI: Confidence Interval
CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration

COX-2: Cyclooxygenase-2
CRP: C-Reactive Protein
CV: Coefficient of Variation
DKD: Diabetic Kidney Disease
DM: Diabetes Mellitus
eGFR: Estimated Glomerular Filtration Rate
ELISA: Enzyme-Linked Immunosorbent Assay
ESRD: End-Stage Renal Disease
FBS: Fasting Blood Sugar
GDF-15: Growth Differentiation Factor-15
HbA1c: Glycated Haemoglobin
HDL: High-Density Lipoprotein
HPLC: High-Performance Liquid Chromatography
IL-6: Interleukin-6
IQR: Interquartile Range
JNK: c-Jun N-terminal Kinase
KDIGO: Kidney Disease: Improving Global Outcomes
LDL: Low-Density Lipoprotein
NF-κB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NPV: Negative Predictive Value
Ntn1: Netrin-1
OR: Odds Ratio
PPV: Positive Predictive Value
RAGE: Receptor for Advanced Glycation End Products
ROC: Receiver Operating Characteristic
SD: Standard Deviation
STROBE: Strengthening the Reporting of Observational Studies in Epidemiology
T2DM: Type 2 Diabetes Mellitus
TGF-β: Transforming Growth Factor-Beta
uACR: Urinary Albumin-to-Creatinine Ratio
Unc5b: Uncoordinated-5-B
VIF: Variance Inflation Factor
WHO: World Health Organisation

Declarations

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Scientific Committee of the Department of Chemistry and Biochemistry, College of Medicine, University of Babylon (document number 5071; approval date 18 September 2025). Informed consent was obtained verbally from all participants after the study's objectives, procedures, risks, and benefits were thoroughly explained in Arabic by a member of the research team, and the consent process was witnessed and documented in the participant's clinical record. Verbal rather than written consent was approved by the Ethics Committee on the basis that this was a minimal-risk observational study involving only routine venepuncture and a midstream urine sample, with no investigational procedure beyond standard care, and given that a portion of recruited participants had limited literacy. This approach is consistent with the Declaration of Helsinki §26 and with local institutional practice for minimal-risk biomarker studies in Iraqi public-hospital settings. A copy of the ethics approval letter is available from the corresponding author upon request.

Consent for Publication

All authors gave consent for the publication of this work under the Creative Commons Attribution Non-Commercial 4.0 license. The manuscript does not contain any person's data, including images or personal details, that could identify any participant. Participants were informed that the results of the study would be published in a peer-reviewed journal without identifying personal information, and all provided verbal consent for this.

Data Availability

The de-identified individual participant data underlying this study, the analytical code (SPSS syntax and R scripts) used for ROC analysis, DeLong testing, and multivariable logistic regression, and the completed STROBE checklist are available from the corresponding author upon reasonable request, conditional on a data-use agreement compliant with the Declaration of Helsinki and approval by the Babylon University Ethics Committee. Requests should be directed to the corresponding author at Husseinallawitami99@gmail.com.

Competing Interests

The authors declare that they have no competing interests. No financial or non-financial relationships exist that could be perceived as influencing the results or interpretation of this study.

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Author Contributions

THA worked on the conceptualisation, data curation, formal analysis, investigation, methodology, project administration, software, validation, visualisation, writing the original draft, writing the review and editing

BHR worked on the conceptualisation, investigation, methodology, resources, supervision, validation, writing of the review and editing.

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REFERENCES

1. Magliano DJ, Boyko EJ, Atlas ID. COVID-19 and diabetes. InIDF DIABETES ATLAS [Internet]. 10th edition. International Diabetes Federation. 2021.
2. Koye DN, Magliano DJ, Nelson RG, Pavkov ME. The global epidemiology of diabetes and kidney disease. Advances in Chronic Kidney Disease. 2018 Mar 1;25(2):121-32. https://doi.org/10.1053/j.ackd.2017.10.011
3. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, Hu FB, Kahn CR, Raz I, Shulman GI, Simonson DC. Type 2 diabetes mellitus. Nature Reviews Disease Primers. 2015 Jul 23;1(1):15019. https://doi.org/10.1038/nrdp.2015.19
4. Hoogeveen EK. The epidemiology of diabetic kidney disease. Kidney and Dialysis. 2022 Aug 1;2(3):433-42. https://doi.org/10.3390/kidneydial2030038
5. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. Clinical journal of the American Society of Nephrology. 2017 Dec 1;12(12):2032-45. https://doi.org/10.2215/CJN.11491116
6. Tonneijck L, Muskiet MH, Smits MM, Van Bommel EJ, Heerspink HJ, Van Raalte DH, Joles JA. Glomerular hyperfiltration in diabetes: mechanisms, clinical significance, and treatment. Journal of the American Society of Nephrology. 2017 Apr 1;28(4):1023-39. https://doi.org/10.1681/ASN.2016060666
7. Gholaminejad A, Fathalipour M, Roointan A. Comprehensive analysis of diabetic nephropathy expression profile based on weighted gene co-expression network analysis algorithm. BMC Nephrology. 2021 Jul 2;22(1):245. https://doi.org/10.1186/s12882-021-02447-2
8. McGill JB, Haller H, Roy-Chaudhury P, Cherrington A, Wada T, Wanner C, Ji L, Rossing P. Making an impact on kidney disease in people with type 2 diabetes: the importance of screening for albuminuria. BMJ Open Diabetes Research & Care. 2022 Jul 5;10(4). https://doi.org/10.1136/bmjdrc-2022-002806
9. Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium?. Diabetologia. 2008 May;51(5):714-25. https://doi.org/10.1007/s00125-008-0961-8
10. Xia X, Hu Z, Wang S, Yin K. Netrin-1: An emerging player in inflammatory diseases. Cytokine & Growth Factor Reviews. 2022 Apr 1;64:46-56. https://doi.org/10.1016/j.cytogfr.2022.01.003
11. Tak E, Ridyard D, Badulak A, Giebler A, Shabeka U, Werner T, Clambey E, Moldovan R, Zimmerman MA, Eltzschig HK, Grenz A. Protective role for netrin-1 during diabetic nephropathy. Journal of Molecular Medicine. 2013 Sep;91(9):1071-80. https://doi.org/10.1007/s00109-013-1041-1
12. Jayakumar C, Nauta FL, Bakker SJ, Bilo H, Gansevoort RT, Johnson MH, Ramesh G. Netrin-1, a urinary proximal tubular injury marker, is elevated early in the time course of human diabetes. Journal of Nephrology. 2014 Apr 1;27(2):151-7. https://doi.org/10.1007/s40620-014-0055-2
13. White JJ, Mohamed R, Jayakumar C, Ramesh G. Tubular injury markers netrin-1 is elevated early in experimental diabetes. Journal of Nephrology. 2013 Aug 7;26(6):1055. https://doi.org/10.5301/jn.5000303
14. Carlsson AC, Nowak C, Lind L, Östgren CJ, Nyström FH, Sundström J, Carrero JJ, Riserus U, Ingelsson E, Fall T, Ärnlöv J. Growth differentiation factor 15 (GDF-15) is a potential biomarker of both diabetic kidney disease and future cardiovascular events in cohorts of individuals with type 2 diabetes: a proteomics approach. Upsala Journal of Medical Sciences. 2020 Jan 2;125(1):37-43. https://doi.org/10.1080/03009734.2019.1696430
15. Lasaad S, Crambert G. GDF15, an emerging player in renal physiology and pathophysiology. International Journal of Molecular Sciences. 2024 May 29;25(11):5956. https://doi.org/10.3390/ijms25115956
16. Zhang X, Wang S, Chong N, Chen D, Shu J, Sun J, Sun Z, Wang R, Wang Q, Xu Y. GDF-15 alleviates diabetic nephropathy via

inhibiting NEDD4L-mediated IKK/NF-κB signalling pathways. International Immunopharmacology. 2024 Feb 15;128:111427. https://doi.org/10.1016/j.intimp.2023.111427
17. Chen J, Peng H, Chen C, Wang Y, Sang T, Cai Z, Zhao Q, Chen S, Lin X, Eling T, Wang X. NAG-1/GDF15 inhibits diabetic nephropathy via inhibiting AGE/RAGE-mediated inflammation signaling pathways in C57BL/6 mice and HK-2 cells. Life Sciences. 2022 Dec 15;311:121142. https://doi.org/10.1016/j.lfs.2022.121142
18. Zhang X, Zhou CG, Ma LJ. Role of GDF-15 in diabetic nephropathy: mechanisms, diagnosis, and therapeutic potential. International Urology and Nephrology. 2025 Jan;57(1):169-75. https://doi.org/10.1007/s11255-024-04179-2
19. Behnoush AH, Khalaji A, Shokri Varniab Z, Rahbarghazi A, Amini E, Klisic A. Urinary and circulatory netrin-1 as biomarker in diabetes and its related complications: a systematic review and meta-analysis. Endocrine. 2024 May;84(2):328-44. https://doi.org/10.1007/s12020-023-03598-y
20. Ay E, Marakoğlu K, Kizmaz M, Ünlü A. Evaluation of Netrin-1 levels and albuminuria in patients with diabetes. Journal of Clinical Laboratory Analysis. 2016 Nov;30(6):972-7. https://doi.org/10.1002/jcla.21965
21. Jung HI, Bae J, Han E, Kim G, Lee JY, Kim SR, Lee BW, Kang ES, Ahn CW, Cha BS, Lee YH. Circulating Netrin-1 as a novel biomarker for impaired fasting glucose and newly diagnosed type 2 diabetes mellitus. Diabetes. 2018 Jul 1;67(Supplement_1):1535-P. https://doi.org/10.2337/db18-1535-P
22. Al-Shakour AA, Khalid HA, Mahmood I, Al-Sudani MY. Serum netrin-1 level in patients with type 2 diabetes mellitus and its relationship with diabetic complications. Anaesthesia, Pain & Intensive Care. 2024 Apr 1;28(2):347.
23. Elkholy RA, Younis RL, Allam AA, Hagag RY, Abdel Ghafar MT. Diagnostic efficacy of serum and urinary netrin-1 in the early detection of diabetic nephropathy. Journal of Investigative Medicine. 2021 Aug;69(6):1189-95. https://doi.org/10.1136/jim-2021-001785
24. Hacıhamdioğlu DÖ, Hacıhamdioğlu B, Altun D, Müftüoğlu T, Karademir F, Süleymanoğlu S. Urinary netrin-1: a new biomarker for the early diagnosis of renal damage in obese children. Journal of Clinical Research in Pediatric Endocrinology. 2016 Sep 1;8(3):282. https://doi.org/10.4274/jcrpe.2828
25. Kamianowska M, Szczepański M, Chomontowska N, Trochim J, Wasilewska A. Is Urinary Netrin-1 a Good Marker of Tubular Damage in Preterm Newborns?. Journal of Clinical Medicine. 2021 Feb 19;10(4):847. https://doi.org/10.3390/jcm10040847
26. Yimer EM, Zewdie KA, Hishe HZ. Netrin as a Novel Biomarker and Its Therapeutic Implications in Diabetes Mellitus and Diabetes-Associated Complications. Journal of Diabetes Research. 2018;2018(1):8250521. https://doi.org/10.1155/2018/8250521
27. Nair V, Robinson-Cohen C, Smith MR, Bellovich KA, Bhat ZY, Bobadilla M, Brosius F, de Boer IH, Essioux L, Formentini I, Gadegbeku CA. Growth differentiation factor–15 and risk of CKD progression. Journal of the American Society of Nephrology. 2017 Jul 1;28(7):2233-40. https://doi.org/10.1681/ASN.2016080919
28. Agarwal RG, Khokhar M, Purohit P, Modi A, Bajpai NK, Bohra GK, Garg MK, Sharma P. A clinical and in-silico study of microRNA-21 and growth differentiation factor-15 expression in pre-diabetes, type 2 diabetes and diabetic nephropathy. Minerva Endocrinology. 2022 Feb 1;48(2):172-85. https://doi.org/10.23736/S2724-6507.22.03646-6