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# Association of serum galectin-3 levels and LGALS3 gene expression across albuminuria categories in patients with type 2 diabetes mellitus: A cross-sectional study

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**Abstract.** *Diabetic kidney disease (DKD) remains the leading cause of end-stage kidney disease worldwide because conventional markers, such as urinary albumin and the estimated glomerular filtration rate, are not sensitive enough to detect renal stress until significant damage has occurred. Galectin-3 (Gal-3), a β-galactoside-binding lectin encoded by the galectin-3 (LGALS3) gene, is implicated in renal fibrosis, macrophage-driven inflammation, and advanced glycation end-product signaling, making it a biologically plausible candidate biomarker.*

*Methods. This cross-sectional study enrolled 90 adults with type 2 diabetes mellitus (T2DM), stratified by urinary albumin-to-creatinine ratio (UACR) into A1 (UACR < 30 mg/g), A2 (UACR 30–300 mg/g), and A3 (UACR > 300 mg/g) categories (n = 30 each), along with 30 age- and sex-matched healthy controls. Serum Gal-3 was measured by sandwich enzyme-linked immunosorbent assay (ELISA), and LGALS3 messenger RNA expression in peripheral blood mononuclear cells (PBMCs) was quantified by reverse transcription quantitative polymerase chain reaction. Discriminative performance was assessed using receiver operating characteristic (ROC) analysis, and the independent statistical association of Gal-3 was evaluated using multivariate logistic regression.*

*Results. Serum Gal-3 was significantly elevated in normoalbuminuric T2DM patients compared to that in healthy controls (21.2 ± 3.6 vs 17.6 ± 3.8 ng/mL; p = 0.003) and rose progressively across albuminuria categories (A1 to A3; one-way analysis of variance [ANOVA] p < 0.001). The LGALS3 mRNA expression increased simultaneously. Serum Gal-3 showed good cross-sectional discriminative ability for any category of albuminuria A2/A3 vs A1; area under the curve 0.84, 95% CI 0.77–0.91, and remained independently associated with albuminuria after multivariate adjustment (odds ratio 1.42 per ng/mL; 95% CI 1.18–1.71; p < 0.001).*

*Conclusion. Serum Gal-3 and LGALS3 expression levels are significantly associated with albuminuria in T2DM and may serve as complementary markers. However, future longitudinal validation studies are required to establish whether Gal-3 has a predictive value for the progression of diabetic kidney disease.*

**Keywords:** *galectin 3, albuminuria, diabetes mellitus type 2, diabetic nephropathies, gene expression.*

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# Зв'язок сироваткової концентрації галектину-3 та експресії гена LGALS3 з категоріями альбумінурії у пацієнтів з цукровим діабетом 2 типу: одномоментне поперечне дослідження

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**Резюме.** *Діабетична хвороба нирок (ДХН) залишається однією з провідних причин термінальної стадії хронічної хвороби нирок в світі. Традиційні маркери, зокрема альбумінурія та розрахункова швидкість клубочкової фільтрації, не завжди дають змогу діагностувати ДХН вчасно. Галектин-3 (Gal-3) – β-галактозидзв'язувальний лектин, кодований геном LGALS3, який бере участь у розвитку фіброзу нирок, макрофаг-опосередкованого запалення та сигнальних шляхах кінцевих продуктів глікування, що обґрунтовує його потенційне значення як біомаркера ураження нирок.*

*Методи. До цього поперечного дослідження включено 90 дорослих пацієнтів з цукровим діабетом 2 типу (ЦД2), яких розподілили відповідно до альбумін-креатинінового співвідношення сечі (АКС) на категорії А1 (АКС < 30 мг/г), А2 (АКС 30–300 мг/г) та А3 (АКС > 300 мг/г), n = 30 в кожній групі. Контрольну групу становили 30 здорових осіб, зіставних за віком і статтю. Сироваткову концентрацію Gal-3 визначали методом твердофазного імуноферментного аналізу. Експресію матричної РНК гена LGALS3 у мононуклеарних клітинах периферичної крові кількісно визначали методом полімеразної ланцюгової реакції в реальному часі зі зворотною транскрипцією. Дискримінаційну здатність Gal-3 оцінювали за допомогою ROC-аналізу. Незалежний зв'язок Gal-3 з альбумінурією оцінювали за допомогою багатофакторного логістичного регресійного аналізу.*

*Результати. Концентрація Gal-3 в сироватці крові була достовірно вищою у пацієнтів з ЦД2 та альбумінурією категорії А1 порівняно зі здоровими особами (21,2 ± 3,6 проти 17,6 ± 3,8 нг/мл; p = 0,003) і поступово зростала від категорії А1 до А3 (p < 0,001 за однофакторним дисперсійним аналізом). Паралельно зростала експресія мРНК гена LGALS3. Gal-3 продемонстрував дискримінаційну здатність щодо наявності альбумінурії категорій А2/А3 порівняно з А1: площа під ROC-кривою становила 0,84 (95% ДІ 0,77–0,91). Після багатофакторної поправки вища концентрація Gal-3 залишалась незалежно асоційованою з наявністю альбумінурії категорій А2/А3 (відношення шансів 1,42 на кожне підвищення Gal-3 на 1 нг/мл; 95% ДІ 1,18–1,71; p < 0,001).*

*Висновки. Концентрація Gal-3 в сироватці крові та експресія гена LGALS3 статистично значущо асоціювалась з вираженістю альбумінурії у пацієнтів з ЦД2 і може бути додатковими біомаркерами ураження нирок. Для визначення прогностичного значення Gal-3 щодо прогресування ДХН необхідні подальші проспективні дослідження.*

**Ключові слова:** *галектин-3, альбумінурія, цукровий діабет 2 типу, діабетична хвороба нирок, експресія генів.*

**Introduction.** More than 537 million adults have type 2 diabetes mellitus (T2DM) worldwide, 30–40% of whom develop diabetic kidney disease (DKD), a leading cause of end-stage kidney disease (ESKD) [1, 2], with Iraq and the wider Middle East and North Africa region among the most affected [4, 5]. Histological confirmation was not performed in this study; therefore, "diabetic kidney disease" was diagnosed according to current Kidney Disease: Improving Global Outcomes (KDIGO) recommendations for clinically defined renal involvement in T2DM, based on urinary albumin-to-creatinine ratio (UACR) and estimated glomerular filtration rate (eGFR) parameters [3].

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Despite their central role in diagnosing and staging DKD, UACR and eGFR have well-recognized limitations: albuminuria may be absent despite substantial renal structural damage, eGFR decline typically occurs late, some patients progress to advanced DKD without an albuminuric stage, and albuminuria itself fluctuates with blood pressure, glycemic control, and medication [3, 6–9]. Supplementary biomarkers able to detect early pathophysiological change are therefore needed.

The LGALS3 gene, located at chromosome 14q21–22, encodes galectin-3 (Gal-3), a β-galactoside-binding lectin [10]. It is expressed across several cell types—macrophages, fibroblasts, epithelial cells, and endothelial cells—where it participates in adhesion, proliferation, apoptosis, inflammation, and fibrotic processes [11, 12]. Within the kidney, injury induces Gal-3 upregulation, and the protein contributes to renal fibrosis by activating transforming growth factor beta-1 (TGF-β1) signaling, recruiting macrophages, and promoting extracellular matrix deposition [13, 14].

In diabetic mouse models, both genetic knockout and pharmacological blockade of Gal-3 have been shown to reduce albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis [15, 16].

Human studies link circulating Gal-3 to prevalent chronic kidney disease (CKD), declining eGFR, and progression to ESKD, in people with and without diabetes [17–19]. Most of this work, however, has been carried out in patients who already have established albuminuria or reduced eGFR; the A1 category, where conventional markers still fall within normal limits, has received little attention. It also remains unclear how circulating Gal-3 relates to LGALS3 mRNA expression in peripheral blood mononuclear cells (PBMCs) in the setting of DKD.

This study set out to examine how serum Gal-3 and PBMC LGALS3 mRNA expression relate to albuminuria status across all categories in Iraqi patients with T2DM. We hypothesized that both serum Gal-3 and LGALS3 expression would be elevated across the albuminuria spectrum, including the A1 category, and that Gal-3 would provide independent discriminative value beyond conventional clinical and biochemical biomarkers.

**Materials and methods.** *Study design and ethical approval*. This single-center, cross-sectional study was conducted at the Diabetes and Endocrinology Center, Baghdad, Iraq, between July 2025 and December 2025. The Middle Technical University Institutional Review Board approved the study protocol (MEC: 230, dated 08 June 2025), and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to enrolment.

*Participant recruitment and flow*. Participants were recruited consecutively from the outpatient department of the Diabetes and Endocrinology Center in Baghdad, Iraq. A total of 178 individuals were screened for eligibility, and 58 patients were excluded based on the exclusion criteria, resulting in a final cohort of 120 participants (**Supplementary Figure S1**). Patient recruitment was performed consecutively to minimize selection bias.

*Inclusion and exclusion criteria*. Inclusion criteria for T2DM patients: age ranged 30–65 years; confirmed diagnosis of T2DM according to American Diabetes Association criteria (duration ≥ 5 years); stable glycemic control (glycated hemoglobin [HbA1c] 7.0–10.5%) for at least 3 months; willingness to provide informed consent.

Inclusion criteria for healthy controls: Age ranged 30–65 years; fasting blood glucose < 5.6 mmol/L and HbA1c < 5.7%; no history of diabetes, hypertension, or chronic kidney disease; UACR < 3 0 mg/g; and eGFR > 90 mL/min/1.73 $m^2$.

Exclusion criteria (applied to all participants): Type 1 diabetes mellitus or secondary diabetes; acute kidney injury or non-diabetic kidney disease; active urinary tract infection or hematuria; known hepatic disease (cirrhosis, chronic hepatitis, alanine aminotransferase [ALT] > 3 times the upper limit of normal); active inflammatory or autoimmune disease; active malignancy or history of malignancy within 5 years; established heart failure (New York Heart Association [NYHA] class II–IV); significant cardiovascular disease (prior myocardial infarction, stroke, or revascularization within 12 months); pregnancy or lactation; use of immunosuppressive therapy or systemic corticosteroids within 3 months.

*Sample size calculation*. Sample size was determined a priori using published data from Chung et al. (2022) [20], who reported a mean difference in serum Gal-3 of approximately 7 ng/mL between T2DM patients with and without albuminuria compared to that in healthy controls (standard deviation [SD] ≈ 6 ng/mL). This translated into a required minimum of 24 participants per group (two-tailed t-test, 80% power, $\alpha = 0.05$). To allow for an anticipated 15–20% attrition and to strengthen subgroup comparisons, 30 patients per group were recruited, giving a total sample of 120 (G*Power version 3.1) [21].

*Study groups*. Participants were stratified into four groups (n = 30 each): (1) healthy controls (no diabetes, UACR < 30 mg/g, eGFR > 90 mL/min/1.73 $m^2$); (2) T2DM with A1 category (UACR < 30 mg/g); (3) T2DM with A2 category (UACR 30–300 mg/g); (4) T2DM with A3 category (UACR > 300 mg/g). UACR classification was based on the mean of two first-morning urine samples collected within a 1-month window, and eGFR was derived using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [22].

*Clinical and biochemical assessment*. Standardized clinical assessment (medical history, physical examination, and anthropometric measurement) was performed for all participants. BMI was derived as weight (kg) divided by height squared ($m^2$). Blood pressure was taken in triplicate after a 10-minute rest with an automated sphygmomanometer, and the second and third readings were averaged. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or current antihypertensive treatment.

Participants were categorized by smoking history as never, ex-smoker (>6 months since quitting), or current smoker. Peripheral neuropathy was assessed clinically using the 10-g monofilament test and vibration perception threshold. Diabetic retinopathy status was determined by fundoscopy wherever this was available; the exam was completed for 86 of 90 T2DM participants (96%), with three A1-group patients lacking a fundoscopy record. All enrolled participants were confirmed free of cardiovascular disease and established heart failure (NYHA class II–IV), which were pre-specified exclusion criteria. Medication history—angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), sodium-glucose cotransporter-2 inhibitors (SGLT2i), metformin, sulfonylureas, insulin, statins, and antiplatelet agents—was obtained through structured interview and cross-checked against pharmacy records where available.

Venous blood samples were collected after an overnight fast (≥ 8 hours). Fasting blood glucose, serum creatinine, urea, uric acid, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, and C-reactive protein (CRP) levels were measured using standard automated enzymatic and immunoturbidimetric assays on a Cobas c501 analyzer (Roche Diagnostics, Mannheim, Germany). HbA1c levels were measured by high-performance liquid chromatography (Bio-Rad Variant II Turbo, Hercules, CA, USA). Fasting insulin was measured by chemiluminescent immunoassay (Siemens Immulite 2000, Erlangen, Germany), and homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as: [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5 [23].

*Serum Gal-3 measurement*. Sera were separated from patient samples for Gal-3 measurement within 30 minutes of venipuncture. Sera were aliquoted and stored at -80 °C for a maximum of 6 months prior to analysis. No sample underwent more than one freeze-thaw cycle. Visibly hemolyzed or lipemic samples were excluded prior to analysis (n = 3). Samples were randomly analyzed with respect to the clinical groups, with laboratory analysts blinded to group allocation throughout the ELISA process.

Serum Gal-3 concentration was measured using a commercially available sandwich ELISA kit (Human Galectin-3 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, USA; catalogue number DGAL30) according to the manufacturer's instructions, with intra- and inter-assay CVs of 3.2% and 5.8%, respectively. All samples were analyzed in duplicate, and the mean values were used in analyses. Full assay parameters (storage duration, dilution protocol, assay range) are provided in Supplementary Methods.

*PBMC isolation and RNA extraction*. PBMCs were isolated from ethylenediaminetetraacetic acid (EDTA)-anticoagulated whole blood by density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) within 2 hours of venipuncture. Isolated PBMCs were washed twice in phosphate-buffered saline, counted, and lysed immediately using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was extracted according to the manufacturer's protocol, treated with DNase I (Thermo Fisher Scientific, Waltham, MA, USA) to remove genomic DNA contamination, and quantified using spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific). RNA purity was assessed by A260/A280 ratio (accepted range 1.9–2.1) and RNA integrity by agarose gel electrophoresis. Samples with an RNA integrity number (RIN) < 7.0, or A260/A280 < 1.9 were excluded (n = 4). RNA samples were stored at -80 °C until RT-qPCR analysis.

*Reverse transcription quantitative PCR*. RT-qPCR was performed following key MIQE recommendations [24]. First-strand complementary DNA (cDNA) was synthesized from 1 μg total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) with random hexamer primers according to the manufacturer's protocol. Reverse transcriptase controls were not included to confirm the absence of genomic DNA contamination.

Quantitative PCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems) using TaqMan Gene Expression Assays for LGALS3, with GAPDH as the reference gene, following standard cycling conditions. No template controls were included for each plate. Full assay IDs, reaction volumes, and cycling parameters are provided in Supplementary Methods.

Amplification efficiency, quantification cycle (Cq) values, and inter-assay CVs for LGALS3 and GAPDH are listed in **Supplementary Table S1**. Laboratory personnel performing RT-qPCR were blinded to clinical group allocation. Relative LGALS3 expression was calculated using the 2^−ΔΔCq method [25], with healthy controls as the reference group. We acknowledge that a single reference gene (GAPDH) was used, which is a limitation of this study; however, GAPDH expression was stable across groups (CV < 5%), consistent with prior studies in this field [26, 27].

*Statistical analysis*. R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria) and SPSS version 26.0 (IBM Corp., Armonk, NY, USA) were used to analyze the data. The Shapiro-Wilk test and visual examination of Q-Q plots were used to assess the normality of continuous variables. One-way ANOVA with Tukey's honestly significant difference (HSD) post hoc test was used to compare normally distributed variables, which are presented as mean ± SD. Non-normally distributed variables are presented as median (interquartile range [IQR]) and were compared using the Kruskal-Wallis test with Dunn's post hoc test. Categorical variables are presented as frequency (percentage) and were compared using the chi-square test or Fisher's exact test, as appropriate.

Bonferroni correction was applied to the four primary, between-group comparisons for Gal-3 and LGALS3 (adjusted $\alpha = 0.0125$). Spearman correlation analyses were treated as exploratory and not corrected for multiple comparisons.

Receiver operating characteristic (ROC) curve analysis was performed to assess the cross-sectional discriminative ability of serum Gal-3 levels for albuminuria (A2 or A3 vs A1) within the T2DM cohort. Youden's index (maximum [sensitivity + specificity −1]) was used to calculate the optimal cutoff. Bootstrap validation with 1,000 iterations was performed to obtain bias-corrected 95% CIs for the area under the curve (AUC). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated at the optimal cutoff.

Multivariate logistic regression was performed to identify independent statistical correlates of albuminuria (A2 or A3 vs A1) within the T2DM cohort. The outcome was either category of albuminuria (60 events) vs A1 (30 non-events). The candidate predictor variables

included serum Gal-3, HbA1c, eGFR, BMI, CRP, systolic blood pressure, and diabetes duration (seven predictors; event-per-variable ratio, approximately 8.6:1). Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test, and discrimination was quantified using Nagelkerke $R^2$. A reduced model, including only the three most clinically relevant predictors (Gal-3, HbA1c, and eGFR), was also fitted for the sensitivity analysis.

The incremental value of adding Gal-3 to the baseline clinical model (HbA1c, eGFR, BMI, and CRP) was assessed by comparing AUCs using the DeLong test [28], with category-free integrated discrimination improvement (IDI) and net reclassification improvement (NRI) calculated using the R package "PredictABEL" [29] and bootstrap resampling (1,000 iterations) for 95% CIs.

All statistical tests were two-tailed. For primary comparisons, $p < 0.0125$ (Bonferroni-corrected) was considered significant. For secondary and exploratory analyses, statistical significance was set at $p < 0.05$. Exact p-values are reported where $p \geq 0.001$; for $p < 0.001$, this is stated explicitly.

**Results.** *Baseline characteristics*. Baseline demographic, clinical, and biochemical characteristics of the study population are shown in Table 1.

*Table 1*

**Baseline demographic, clinical, and biochemical characteristics of study participants**

| Parameter | Healthy Controls (n=30) | T2DM A1 (n=30) | T2DM A2 (n=30) | T2DM A3 (n=30) | p-value |
|---|---|---|---|---|---|
| Age (years) | 42 ± 9 | 44 ± 10 | 46 ± 11 | 47 ± 12 | 0.42 |
| Sex (male), n (%) | 17 (57) | 16 (53) | 17 (57) | 18 (60) | 0.68 |
| Diabetes duration (years) | – | 8 ± 3 | 11 ± 4 | 14 ± 5 | <0.001 |
| BMI (kg/m$^2$) | 23.4 ± 2.8 | 28.6 ± 3.4 | 30.2 ± 3.8 | 31.8 ± 4.1 | <0.001 |
| Systolic BP (mmHg) | 118 ± 9 | 132 ± 11 | 142 ± 13 | 152 ± 14 | <0.001 |
| Diastolic BP (mmHg) | 76 ± 6 | 82 ± 8 | 88 ± 9 | 94 ± 10 | <0.001 |
| Hypertension, n (%) | 0 (0) | 13 (43) | 19 (63) | 26 (87) | <0.001 |
| Smoking (current), n (%) | 6 (20) | 7 (23) | 8 (27) | 9 (30) | 0.73 |
| Peripheral neuropathy, n (%) | 0 (0) | 7 (23) | 14 (47) | 21 (70) | 0.002 |
| Diabetic retinopathy, n (%) | 0 (0) | 4/27 (15) | 11/29 (38) | 18/30 (60) | <0.001 |
| CVD history, n (%) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | N/A |
| Heart failure (NYHA II–IV), n (%) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | N/A |
| FBS (mmol/L) | 5.1 ± 0.4 | 8.9 ± 1.6 | 9.8 ± 1.9 | 10.6 ± 2.1 | <0.001 |
| HbA1c (%) | 5.2 ± 0.3 | 7.8 ± 0.9 | 8.6 ± 1.1 | 9.4 ± 1.3 | <0.001 |
| HOMA-IR | 1.8 ± 0.6 | 4.2 ± 1.3 | 5.1 ± 1.6 | 5.8 ± 1.9 | <0.001 |
| Total cholesterol (mmol/L) | 4.6 ± 0.8 | 5.2 ± 1.1 | 5.4 ± 1.2 | 5.6 ± 1.3 | 0.003 |
| LDL-C (mmol/L) | 2.7 ± 0.6 | 3.2 ± 0.9 | 3.4 ± 1.0 | 3.6 ± 1.1 | 0.002 |
| HDL-C (mmol/L) | 1.3 ± 0.3 | 1.1 ± 0.2 | 1.0 ± 0.2 | 0.9 ± 0.2 | <0.001 |
| Triglycerides (mmol/L) | 1.4 ± 0.5 | 2.1 ± 0.8 | 2.4 ± 0.9 | 2.7 ± 1.0 | <0.001 |
| Serum creatinine (μmol/L) | 75 ± 12 | 82 ± 14 | 96 ± 18 | 124 ± 26 | <0.001 |

*Continuation of Table 1*

| Parameter | Healthy Controls (n=30) | T2DM A1 (n=30) | T2DM A2 (n=30) | T2DM A3 (n=30) | p-value |
|---|---|---|---|---|---|
| Urea (mmol/L) | 5.2 ± 1.1 | 6.1 ± 1.4 | 7.8 ± 1.9 | 10.4 ± 2.8 | <0.001 |
| Uric acid (μmol/L) | 298 ± 52 | 342 ± 68 | 378 ± 74 | 412 ± 86 | <0.001 |
| eGFR (mL/min/1.73 m²) | 98 ± 8 | 91 ± 12 | 76 ± 14 | 58 ± 16 | <0.001 |
| UACR (mg/g) [median, IQR] | 12 [8–18] | 16 [10–22] | 98 [52–148] | 586 [312–892] | <0.001 |
| CRP (mg/L) | 1.2 ± 0.6 | 3.8 ± 1.4 | 5.2 ± 1.9 | 6.8 ± 2.3 | <0.001 |

*Data are presented as mean ± SD or n (%). BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; FBS, fasting blood sugar; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; MI, myocardial infarction; NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; UACR, urinary albumin-to-creatinine ratio; CRP, C-reactive protein; revasc., revascularisation; T2DM, type 2 diabetes mellitus; A1, UACR <30 mg/g; A2, UACR 30–300 mg/g; A3, UACR >300 mg/g. p-values from one-way ANOVA (continuous variables) or chi-square test (categorical variables). Post hoc comparisons (Tukey's HSD): p < 0.0125 vs healthy controls; p < 0.0125 vs A1; p < 0.0125 vs A2. †Diabetic retinopathy assessed by fundoscopy; denominator reflects participants with available fundoscopy data (A1: n=27/30; A2: n=29/30; A3: n=30/30); three A1 participants had no fundoscopy record and are excluded from this row only. ‡Significant CVD (prior MI, stroke, or revascularisation within 12 months) and established heart failure (NYHA class II–IV) were pre-specified exclusion criteria; all enrolled participants were confirmed free of these conditions.*

The four groups were well matched for age and sex distribution (p = 0.42 and p = 0.68, respectively). As expected, patients with T2DM had significantly higher BMI, fasting blood glucose, HbA1c, HOMA-IR, CRP, and systolic and diastolic blood pressure, and lower eGFR than healthy controls (p < 0.001 for each of the parameters tested). Within the T2DM cohort, BMI, HbA1c, systolic blood pressure, CRP, and serum creatinine increased progressively across the albuminuria categories (A1–A3), whereas eGFR declined (p < 0.001 for each of the parameters tested).

Prevalence of hypertension increased from 43% in the A1 to 87% in the A3 category (p < 0.001). Peripheral neuropathy was present in 23% of A1, 47% of A2, and 70% of A3 category (p = 0.002). Diabetic retinopathy was present in 15% of A1 (4/27), 38% of A2 (11/29), and 60% of A3 (18/30) category (p < 0.001), consistent with progressive microvascular involvement across the albuminuria categories. No enrolled participant had established heart failure (NYHA class II–IV) or significant cardiovascular disease, as these were pre-specified exclusion criteria. Medication use was similarly distributed among patients with T2DM (**Supplementary Table S2**).

*Serum Gal-3 and LGALS3 mRNA expression*. Serum Gal-3 and LGALS3 mRNA expression levels in PBMCs are shown in Table 2.

*Table 2*

**Serum Gal-3 and LGALS3 mRNA expression across study groups**

| Parameter | Healthy Controls (n=30) | T2DM A1 (n=30) | T2DM A2 (n=30) | T2DM A3 (n=30) | p-value (ANOVA) |
|---|---|---|---|---|---|
| Serum Gal-3 (ng/mL) | 17.6 ± 3.8 | 21.2 ± 3.6[a] | 25.4 ± 4.2[ab] | 30.1 ± 4.6[abc] | <0.001 |
| LGALS3 mRNA (fold change) | 1.00 (reference) | 1.8 ± 0.6[a] | 2.6 ± 0.8[ab] | 3.8 ± 1.2[abc] | <0.001 |

*Data are presented as mean ± SD. Gal-3, galectin-3; LGALS3, lectin galactoside-binding soluble 3 gene; T2DM, type 2 diabetes mellitus; A1, UACR <30 mg/g; A2, UACR 30–300 mg/g; A3, UACR >300 mg/g. LGALS3 mRNA expression is presented as fold change relative to healthy controls, calculated by the 2^−ΔΔCq method. Post hoc comparisons (Tukey's HSD): [a] p < 0.0125 vs healthy controls; [b] p < 0.0125 vs A1; [c] p < 0.0125 vs A2.*

Serum Gal-3 was significantly elevated in normoalbuminuric patients with T2DM (A1 category) compared with healthy controls, and increased progressively across albuminuria categories (one-way ANOVA p < 0.001). Post hoc comparisons showed statistically significant differences between all groups, pairwise (p < 0.0125, Bonferroni-corrected), as illustrated in Figure 1.

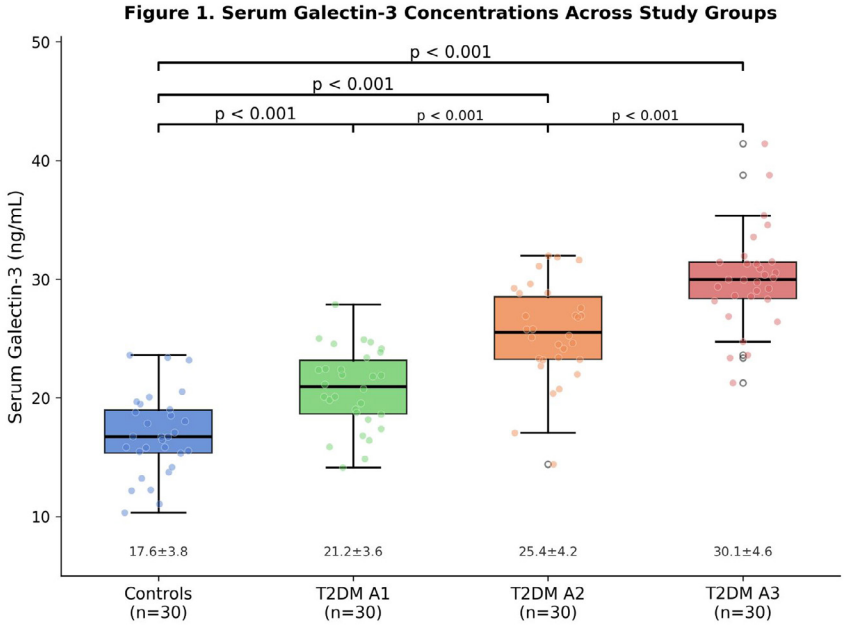


**Fig. 1. Serum Gal-3 concentrations across study groups.** *Box plots showing serum Gal-3 (ng/mL) in healthy controls (n=30), T2DM A1 (n=30), T2DM A2 (n=30), and T2DM A3 (n=30). Boxes represent interquartile range (IQR), horizontal lines represent the median, whiskers extend to 1.5× IQR, and individual points represent outliers. One-way ANOVA p < 0.001. Post hoc comparisons (Tukey's HSD): * p < 0.0125 vs healthy controls; † p < 0.0125 vs A1; ‡ p < 0.0125 vs A2. T2DM, type 2 diabetes mellitus; A1, UACR <30 mg/g; A2, UACR 30–300 mg/g; A3, UACR >300 mg/g; Gal-3, galectin-3.*

LGALS3 mRNA expression in PBMCs increased in a similar pattern across albuminuria categories relative to healthy controls (one-way ANOVA, p < 0.001; Fig. 2). All post hoc pairwise comparisons were statistically significant (p < 0.0125).

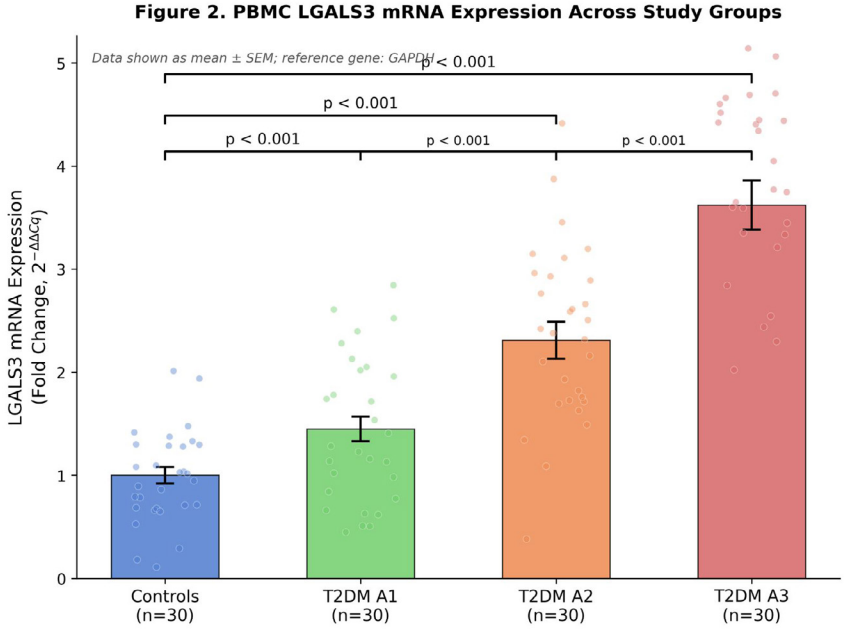


**Fig. 2. PBMC LGALS3 mRNA expression across study groups.** *Bar graph showing LGALS3 mRNA expression (fold change relative to healthy controls) in healthy controls (n=30), T2DM A1 (n=30), T2DM A2 (n=30), and T2DM A3 (n=30). Data are presented as mean ± SD. One-way ANOVA p < 0.001. Post hoc comparisons (Tukey's HSD): * p < 0.0125 vs healthy controls; † p < 0.0125 vs A1; ‡ p < 0.0125 vs A2. PBMC, peripheral blood mononuclear cell; LGALS3, lectin galactoside-binding soluble 3 gene; T2DM, type 2 diabetes mellitus; A1, UACR <30 mg/g; A2, UACR 30–300 mg/g; A3, UACR >300 mg/g.*

*Correlation analyses*. Spearman's correlation analyses between serum Gal-3 levels and clinical and biochemical variables in patients with T2DM (n=90) are presented in Table 3. Serum Gal-3 showed the strongest positive correlation with UACR, followed by LGALS3 mRNA, serum creatinine, CRP, HbA1c, and diabetes duration, and a significant negative correlation with eGFR (all p < 0.001). These correlation analyses were exploratory and were not corrected for multiple comparisons.

*Table 3*

**Spearman correlation of serum Gal-3 with clinical and biochemical parameters in T2DM patients (n=90)**

| Parameter | Correlation coefficient (ρ) | p-value |
|---|---|---|
| UACR, mg/g | 0.76 | <0.001 |
| HbA1c, % | 0.58 | <0.001 |
| Diabetes duration, years | 0.52 | <0.001 |
| BMI, kg/m$^2$ | 0.48 | <0.001 |
| Systolic BP, mmHg | 0.51 | <0.001 |
| eGFR, mL/min/1.73 m$^2$ | –0.68 | <0.001 |
| Serum creatinine, mg/dL | 0.64 | <0.001 |
| CRP, mg/L | 0.62 | <0.001 |
| LGALS3 mRNA expression, relative units | 0.71 | <0.001 |

*UACR, urinary albumin-to-creatinine ratio; HbA1c, glycated haemoglobin; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; LGALS3, lectin galactoside-binding soluble 3 gene. All correlations are exploratory and not corrected for multiple comparisons.*

*Discriminative performance of serum Gal-3*. ROC curve analysis demonstrated good discriminative performance of serum Gal-3 for distinguishing patients with T2DM and albuminuria (microalbuminuria [A2 or A3]) from those with A1 category (AUC, 0.84; 95% CI, 0.77–0.91). The optimal cutoff of 23.5 ng/mL yielded a sensitivity of 78% and specificity of 83% (PPV, 88%; NPV, 71%). Against healthy controls, the AUC was 0.91 (95% CI, 0.86–0.96), with an optimal cutoff of 20.5 ng/mL (sensitivity 85%, specificity 87%, PPV 89%, and NPV 83%). The ROC curves are shown in Figure 3.

**Figure 3. Incremental ROC Analysis of Serum Galectin-3 for Predicting Albuminuria in T2DM Patients**

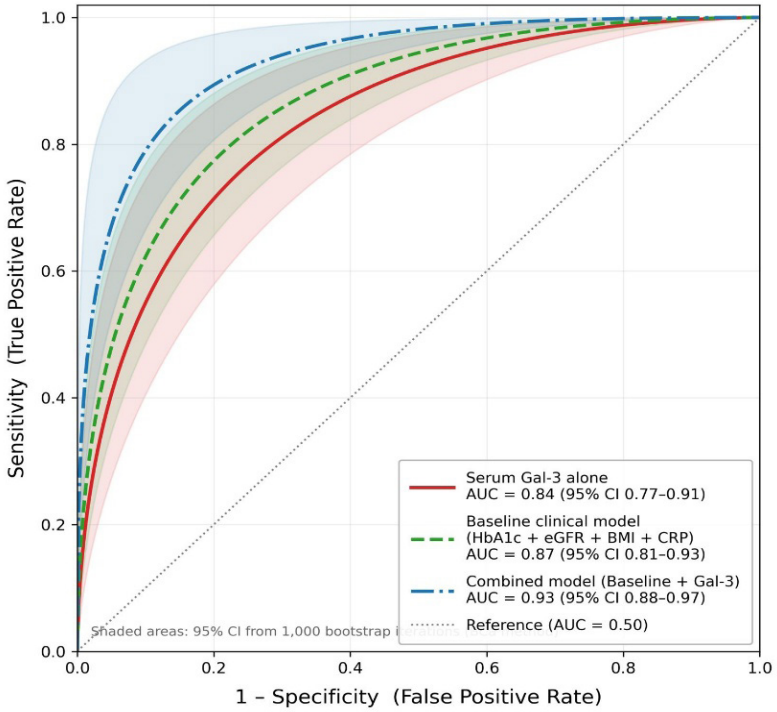


**Fig. 3. ROC curves for discriminating albuminuria (A2/A3 vs A1) in T2DM patients.** *Three ROC curves are shown: (1) Serum Gal-3 alone (AUC 0.84, 95% CI 0.77–0.91); (2) Baseline clinical model (HbA1c, eGFR, BMI, CRP; AUC 0.87, 95% CI 0.81–0.93); (3) Combined model (baseline model + Gal-3; AUC 0.93, 95% CI 0.88–0.97). Addition of Gal-3 to baseline model: ΔAUC = 0.06, DeLong test p = 0.012; IDI 0.08 (95% CI 0.03–0.14, p = 0.002); NRI 0.31 (95% CI 0.12–0.49, p = 0.004). ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; Gal-3, galectin-3; HbA1c, glycated haemoglobin; eGFR, estimated glomerular filtration rate; BMI, body mass index; CRP, C-reactive protein; IDI, integrated discrimination improvement; NRI, net reclassification improvement; T2DM, type 2 diabetes mellitus; A1, UACR <30 mg/g; A2, UACR 30–300 mg/g; A3, UACR >300 mg/g.*

Adding Gal-3 to the baseline clinical model (HbA1c, eGFR, BMI, CRP) significantly improved AUC from 0.87 to 0.93 (DeLong test, p = 0.012), with significant IDI of 0.08 (95% CI, 0.03–0.14; p = 0.002) and NRI of 0.31 (95% CI, 0.12–0.49; p = 0.004). These metrics represent cross-sectional discrimination and do not establish prospective predictive validity.

*Multivariate logistic regression*. Multivariate logistic regression results are presented in Table 4.

*Table 4*

**Multivariate logistic regression for albuminuria (A2/A3 vs A1) in T2DM patients (n=90). Model fit: Hosmer-Lemeshow $\chi^2 = 6.82$, p = 0.56; Nagelkerke $R^2 = 0.61$.**

| Predictor | Odds Ratio (95% CI) | p-value |
|---|---|---|
| Serum Gal-3 (per ng/mL) | 1.42 (1.18–1.71) | <0.001 |
| HbA1c (per %) | 1.68 (1.21–2.34) | 0.002 |
| eGFR (per mL/min/1.73 m$^2$) | 0.92 (0.88–0.96) | <0.001 |
| BMI (per kg/m$^2$) | 1.08 (0.96–1.22) | 0.19 |
| CRP (per mg/L) | 1.12 (0.98–1.28) | 0.09 |
| Systolic BP (per mmHg) | 1.02 (0.98–1.06) | 0.31 |
| Diabetes duration (per year) | 1.06 (0.96–1.17) | 0.24 |

*Gal-3, galectin-3; HbA1c, glycated haemoglobin; eGFR, estimated glomerular filtration rate; BMI, body mass index; CRP, C-reactive protein; BP, blood pressure; CI, confidence interval; T2DM, type 2 diabetes mellitus.*

With all covariates included, serum Gal-3 remained an independent predictor of albuminuria (OR, 1.42 per ng/mL; 95% CI, 1.18–1.71; p < 0.001). Other independent predictors were HbA1c (OR 1.68 per %; 95% CI, 1.21–2.34; p = 0.02) and eGFR (OR, 0.92 per mL/min/1.73 m$^2$; 95% CI, 0.88–0.96; p < 0.001). The model showed adequate calibration (Hosmer-Lemeshow $\chi^2 = 6.82$; p = 0.56) and good discrimination ability (Nagelkerke $R^2 = 0.61$). Findings held up in a reduced model restricted to the three most clinically relevant predictors (Gal-3, HbA1c, and eGFR), supporting the robustness of the primary model (**Supplementary Table S3**).

**Discussion.** This cross-sectional study demonstrated that serum Gal-3 and LGALS3 mRNA levels in PBMC were significantly associated with albuminuria in patients with T2DM in Iraq. Importantly, serum Gal-3 levels were significantly elevated in normoalbuminuric patients with T2DM (A1 category) compared to healthy controls, and increased progressively across all albuminuria categories (A1 to A3). Serum Gal-3 showed good discriminative performance (AUC = 0.84) for distinguishing patients with T2DM and albuminuria (microalbuminuria [A2 or A3]) from those in the A1 category and added an independent statistical association beyond HbA1c and eGFR in the multivariate analysis. These findings suggest that Gal-3 may serve as a complementary biomarker in T2DM; however, longitudinal studies are required to establish its predictive validity for the progression of DKD.

Substantial experimental evidence supports the biological plausibility of Gal-3 as a marker of DKD. In diabetic kidneys, Gal-3 upregulation drives renal fibrosis via TGF-β1 signaling, macrophage recruitment, and extracellular matrix deposition [**13, 14**], and both genetic knockout and pharmacological blockade of Gal-3 have reduced albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis in diabetic mouse models [**15, 16**]. Gal-3 also interacts with advanced glycation end products (AGEs) that build up in diabetes and drive oxidative stress and inflammatory signaling [**30, 31**]. Clinically, circulating Gal-3 has been linked to CKD prevalence, eGFR decline, and progression to ESKD in patients with and without diabetes [**17–19**], and a subcohort analysis from the DECLARE-TIMI 58 trial found that baseline Gal-3 predicted worsening kidney function over a 4.2-year median follow-up in T2DM [**32**]. However, this body of evidence largely derives from patients who already had albuminuria or reduced eGFR; the A1 category, where conventional markers remain normal, has rarely been studied.

Our central finding is that serum Gal-3 was significantly higher in normoalbuminuric T2DM patients (A1 category) than in healthy controls (21.2 ± 3.6 vs 17.6 ± 3.8 ng/mL; p = 0.003), echoing prior reports in T2DM without overt kidney disease [**33, 34**]. This comparison has an important caveat, though: the two groups differed not only in kidney status but also in glycemia, BMI, blood pressure, and CRP—factors independently known to affect circulating Gal-3. The elevated Gal-3 seen in normoalbuminuric T2DM patients could therefore reflect broader diabetes-related inflammation, metabolic stress, or subclinical cardiovascular remodeling rather than early kidney injury specifically. Gal-3 stayed independently associated with albuminuria after our multivariate model adjusted for several of these factors, but residual confounding cannot be ruled out, and this cross-sectional design does not allow us to assume the findings are renal-specific.

Gal-3 is a pleiotropic protein involved in fibrosis, inflammation, and remodeling across multiple organ systems, and is elevated independent of renal disease in

conditions including heart failure, hepatic fibrosis, obesity, hypertension, and subclinical cardiovascular disease [31, 35, 36]. Although patients with known hepatic, inflammatory, and significant cardiovascular conditions were excluded, several of these extra-renal contributors were present to a degree in our cohort: BMI and systolic blood pressure were both higher in T2DM patients and positively correlated with Gal-3 ($\rho = 0.48$ and $\rho = 0.51$, respectively; both $p < 0.001$), yet Gal-3 remained independently associated with albuminuria after adjustment for these factors. Medications such as RAAS blockade (ACEi/ARB) and SGLT2 inhibitors may also influence Gal-3 levels [36], but medication use was distributed similarly across T2DM subgroups. Residual confounding by subclinical myocardial remodeling and other extra-renal sources cannot be fully excluded, and future studies should incorporate cardiac biomarkers and imaging (e.g., echocardiography) to disentangle renal and cardiac contributions to the observed Gal-3 increase.

PBMC LGALS3 mRNA was significantly higher in every T2DM group than in healthy controls and rose progressively with albuminuria severity, pointing to activation of systemic immune-cell LGALS3 transcription that tracks albuminuria in T2DM. That said, PBMC LGALS3 expression reflects circulating immune-cell activity shaped by the inflammatory environment of T2DM and DKD, and it cannot be treated as equivalent to renal parenchymal LGALS3 expression; tissue-level quantification from renal biopsy would be needed to confirm whether the PBMC signal mirrors intrarenal Gal-3 biology. The strong correlation we observed between serum Gal-3 protein and PBMC LGALS3 mRNA ($\rho = 0.71$, $p < 0.001$) is consistent with circulating immune cells being a major contributor to serum Gal-3 in T2DM, in line with the established link between macrophages and Gal-3-driven fibrosis [13, 14]. Other sources, such as adipose tissue, endothelium, and renal parenchyma, likely contribute as well, though we could not quantify their relative contributions here.

For separating T2DM patients with any degree of albuminuria (A2 or A3) from those in the A1 category, serum Gal-3 discriminated well on cross-sectional data (AUC 0.84, 95% CI 0.77–0.91), with sensitivity and specificity at least matching figures reported for other novel biomarkers such as KIM-1 and NGAL in comparable cohorts [36-45]. Adding Gal-3 to a baseline clinical model (HbA1c, eGFR, BMI, and CRP) raised the AUC from 0.87 to 0.93 ($p = 0.012$), with significant IDI and NRI—evidence of incremental cross-sectional value over conventional markers, though as noted, only longitudinal data can establish whether baseline Gal-3 actually predicts incident albumi nuria, eGFR decline, or ESKD. Because it is measured by ELISA, serum Gal-3 is relatively simple and standardized, which could make it useful in settings without access to more complex diagnostics. The RT-qPCR-based LGALS3 mRNA assay described here, by contrast, needs specialist laboratory infrastructure and is not practical for routine or resource-limited use; we therefore regard the LGALS3 findings as primarily mechanistic rather than a candidate clinical test.

These results are consistent with previous observations that patients with T2DM and albuminuria have higher levels of circulating Gal-3 than healthy controls [20, 26, 27]. Chu et al. (2022) reported serum Gal-3 levels of 18.4 ng/mL in normoalbuminuric T2DM patients and 25.6 ng/mL in microalbuminuric patients, consistent with our findings [20]. Tan et al. reported Spearman correlations between Gal-3 and UACR ($r = 0.68$) and eGFR ($r = -0.54$) in a Chinese T2DM cohort, similar to those observed in the present study [26]. Drechsler et al. demonstrated that Gal-3 was an independent predictor of progression to ESKD over a median follow-up of 3.2 years in T2DM patients with CKD stage 2–4 [18]. This work extends the existing literature in several respects: it compares Gal-3 across all three KDIGO albuminuria categories (A1, A2, A3) against matched healthy controls, quantifies circulating PBMC LGALS3 mRNA alongside serum Gal-3, formally evaluates incremental discriminative value through IDI and NRI, and adjusts for a broader set of potential confounders (BMI, CRP, blood pressure, diabetes duration) than most prior studies.

Several limitations should be borne in mind when interpreting these findings. Because the design is cross-sectional, it cannot establish causality or track longitudinal renal outcomes; whether elevated Gal-3 in normoalbuminuric T2DM patients actually predicts later development of albuminuria or eGFR decline is a question only prospective, longitudinal studies can answer. The single-center recruitment setting, a tertiary center in Baghdad, may also limit how well these findings generalize to other populations, healthcare settings, and ethnic groups. The modest sample size of 30 participants per group and 60 events for logistic regression, yielding an events-per-variable ratio of approximately 8.6:1, is at the lower limit of the conventional recommendation; the reduced model sensitivity analysis (**Supplementary Table S3**) confirmed robustness, but larger studies are needed to confirm these findings.

DKD classification was based on clinical criteria (UACR and eGFR) without histological confirmation, as a renal biopsy was not performed. Some participants may have had nondiabetic kidney disease, and the degree of renal fibrosis could not be directly assessed. The diagnostic performance estimates have not been validated in an independent cohort and may be optimistic because of overfitting. Despite multivariate adjustment, unmeasured confounders including dietary factors, physical activity, genetic variants, and subclinical cardiovascular disease may have influenced the association between Gal-3 and albuminuria. Furthermore, Gal-3 is elevated in multiple conditions (heart failure, hepatic fibrosis, obesity, and systemic inflammation), and the renal specificity of these findings could not be definitively established in this study.

PBMC LGALS3 mRNA reflects systemic immune cell transcription and cannot be equated with renal parenchymal expression. Renal biopsy with tissue-level quantification is required to establish intrarenal Gal-3 biology. Medications including ACEi/ARB, SGLT2i, and statins may influence Gal-3 levels and albuminuria. While medication use was distributed similarly across T2DM subgroups, residual confounding factors cannot be excluded. Only one reference gene (GAPDH) was used for RT-qPCR normalization, which, while stable across groups (CV <5%), is a recognized limitation. The use of multiple reference genes would have been preferable. Finally, this study did not assess hard renal outcomes (eGFR decline ≥40%, doubling of serum creatinine levels, ESKD, or renal death) or cardiovascular outcomes, which are the ultimate endpoints of clinical interest.

**Conclusions.** This cross-sectional study demonstrated that serum Gal-3 and PBMC LGALS3 mRNA expression are significantly associated with albuminuria categories in patients with T2DM, including the A1 category, where conventional biomarkers remain within normal limits. Serum Gal-3 demonstrated a good discriminative performance (AUC 0.84) and an independent statistical association with albuminuria beyond HbA1c and eGFR. These findings indicate that Gal-3 may be a complementary marker associated with albuminuria in T2DM; clinical utility has not yet been established. Future studies in larger multi-center cohorts are warranted to assess whether baseline Gal-3 has predictive value for incidence of DKD progression and whether clinically relevant thresholds can be established through longitudinal validation.

**Supplementary materials.** Supplementary Tables and Figures are available on Figshare: **https://doi.org/10.6084/m9.figshare.33311775**.

**Ethics statement.** The study protocol was reviewed and approved by the Middle Technical University Institutional Review Board (MEC: 230, dated 08 June 2025).

**Conflict of interest statement:** The authors declare no conflicts of interest.

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**Availability of data and materials.** The datasets generated and analyzed during this study are available from the corresponding author on reasonable request, subject to ethical and institutional data-sharing restrictions.

**Author contributions.**

***Sama Al-Shaheeb:*** conceived and designed the study, recruited participants, performed all laboratory analyses, conducted all statistical analyses, and wrote the manuscript in its entirety.

***Athir Kadhim Mohammed:*** provided academic supervision and obtained ethical approval, reviewed and approved the final manuscript. Both authors read and approved the submitted version.

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**Artificial intelligence use statement.** No artificial intelligence tools were used in the preparation, writing, analysis, or interpretation of this manuscript. All content was generated by the authors.

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