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**ORIGINAL ARTICLE**

# Evaluation of Serum Levels of Uromodulin, Neutrophil Gelatinase-Associated Lipocalin, and CD4+ T Cell Percentages as Diagnostic Biomarkers in Diabetic Nephropathy

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**ABSTRACT**

Diabetic nephropathy (DN) remains the leading cause of end-Diabetic nephropathy (DN) remains the leading cause of end-stage renal disease worldwide. Early detection is challenging as conventional markers only become abnormal after significant kidney damage has occurred. Uromodulin (UMOD) is a highly organ-specific marker of tubular integrity. Neutrophil Gelatinase-Associated Lipocalin (NGAL) is released from tubular epithelial cells within hours of injury, providing an early signal of tubular stress. CD4+ T-cell activation may contribute to disease progression through pro-inflammatory cytokines and promotion of kidney tissue injury. The study aimed to evaluate serum UMOD, NGAL, and CD4+ T-cell percentages as potential biomarkers for DN. A case-control study was conducted between September 2025 and April 2026 at Al-Sadr Teaching Hospital and Al-Hakim General Hospital, Najaf, Iraq, enrolling 60 healthy controls, 30 T2DM patients, and 30 DN patients. Serum UMOD and NGAL were quantified by sandwich ELISA. CD4+ T-cell percentages were determined by flow cytometry. Diagnostic performance was assessed by ROC curve analysis. Serum UMOD and NGAL were significantly elevated in T2DM and DN compared to controls ($P < 0.001$). CD4+ T-cell percentages were significantly higher in T2DM and DN patients than in controls ($P < 0.001$). ROC analysis showed excellent-to-perfect discrimination (AUC = 0.802–1.000) for all biomarkers; serum NGAL achieved the highest AUC values (0.995 for T2DM; 1.000 for DN). Serum UMOD, NGAL, and CD4+ T-cell percentages were significantly increased in T2DM and DN patients and showed strong diagnostic performance. These markers may serve as useful noninvasive tools for the early detection and monitoring of DN.

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## 1- INTRODUCTION

Diabetes mellitus has become one of the defining chronic disease burdens of the twenty-first century. The International Diabetes Federation estimates that 537 million adults were living with diabetes in 2021, a figure projected to reach 783 million by 2045, with the Middle East and North Africa carrying one of the highest age-standardised prevalence rates globally. Iraq is among the most severely affected countries in the region, with national prevalence estimates consistently reported between 16.8 and 19.7% [1]. Behind these figures lies an

enormous clinical toll, driven by the long-term microvascular and macrovascular complications that develop when glycaemic control remains suboptimal over years and decades. Among the microvascular complications of type 2 diabetes mellitus (T2DM), diabetic nephropathy remains the most clinically consequential complication, accounting for 30 to 50 percent of all new dialysis initiations each year worldwide [2]. The conventional diagnostic strategy relies on urinary albumin-to-creatinine ratio (UACR) and estimated glomerular filtration rate (eGFR); yet this approach identifies renal injury only after irreversible structural damage has already occurred [3]. A substantial proportion of T2DM patients, estimated at 20 to 40 percent, exhibit declining kidney function while remaining normoalbuminuric, and therefore completely invisible to current screening. This diagnostic gap has created an urgent need for earlier, more sensitive molecular biomarkers capable of detecting the subclinical tubular dysfunction that begins years before conventional parameters change [4].

The proximal tubule and the thick ascending limb of the loop of Henle have emerged as the primary sites of early diabetic renal injury. Both segments are particularly vulnerable to hyperglycaemia-driven metabolic stress, oxidative damage, and mitochondrial dysfunction, which collectively erode tubular cell mass and function long before glomerular damage becomes measurable [5]. Two tubular proteins are especially well positioned to serve as indicators of this process: Uromodulin (UMOD) and Neutrophil Gelatinase-Associated Lipocalin (NGAL) [6].
Uromodulin (UMOD), also known as Tamm-Horsfall protein, is a 69-kDa glycoprotein (95-100 kDa after full glycosylation) , synthesised exclusively by epithelial cells of the thick ascending limb of Henle's loop and the early distal tubule. It is the most abundant protein in normal urine, where it plays critical roles in tubular defense against urinary tract infection, regulation of NKCC2 co-transporter activity, and protection of the collecting duct epithelium from crystalline aggregation [7]. A small fraction of newly synthesised UMOD is constitutively released from the basolateral membrane into the interstitium and circulation, and this circulating pool directly reflects the size and metabolic activity of the viable thick ascending limb cell mass , When hyperglycemia, hypoxia, or inflammation progressively destroy these cells, the balance between constitutive UMOD release and tubular cell loss becomes progressively disrupted, and circulating UMOD levels reflect the evolving metabolic and structural state of the thick ascending limb across disease stages [8]. Notably, several studies have documented an initial rise in serum UMOD in early T2DM, attributed to compensatory tubular hypertrophy and increased UMOD secretion under hyperglycaemic conditions, before levels ultimately fall as nephropathy progresses and tubular mass is irreversibly lost, this stage-dependent pattern makes serum UMOD a sensitive and mechanistically grounded indicator of tubular involvement across the T2DM-to-DN continuum [9].

Neutrophil Gelatinase-Associated Lipocalin (NGAL), also known as lipocalin-2 or siderocalin, is a 25-kDa member of the lipocalin superfamily that is constitutively expressed at low levels in tubular epithelial cells, neutrophils, and several other cell types, but is dramatically upregulated, by 10 to 100-fold, within two to six hours of any significant tubular cell stress or injury [10]. In the diabetic kidney, where tubular cells are continuously exposed to glucose toxicity, advanced glycation end-product-mediated oxidative stress, and mitochondrial dysfunction, NGAL is chronically upregulated even at the early T2DM stage, reflecting the ongoing low-grade tubular injury that precedes macroscopic damage [11]. The molecular basis for NGAL upregulation involves NF-κB-mediated transcriptional activation and interleukin-1β-driven signalling in injured tubular cells [12]. As nephropathy progresses, both the amplitude and chronicity of NGAL upregulation increase, providing a biomarker signal that tracks the severity of ongoing tubular injury rather than simply reflecting cumulative cell loss, thereby offering complementary temporal information to the UMOD trajectory [13]. Beyond tubular biomarkers, accumulating evidence indicates that T2DM is accompanied by significant dysregulation of adaptive immunity, particularly of CD4+ T helper cells. CD4+ T cells orchestrate both the inflammatory and fibrotic responses that drive progressive renal injury, and their circulating numbers reflect the balance between hyperglycaemia-driven immune suppression and the pro-inflammatory activation that sustains tubular damage . Chronic hyperglycaemia impairs T cell receptor signaling, reduces CD4+ T cell proliferative capacity through mitochondrial dysfunction, and expands myeloid-derived suppressor cell populations that further constrain $CD4^+$ effector responses [14]. Because the magnitude of CD4+ suppression may differ across disease stages, assessing $CD4^+$ T cell percentages alongside tubular biomarkers could add an immunological dimension to the early detection of diabetic renal injury [15].The present study aims to investigate serum UMOD.NGAL and $CD4^+$ to evaluate their ability to reflect disease severity and identify renal impairment in diabetic kidney disease.

## 2- MATERIALS AND METHODS

This study was approved by the ethics committees of Al-Sadr Teaching Hospital, Al-Hakim General Hospital in Najaf, and the College of Medicine, University of Al-Qadisiyah. Written informed consent was obtained from each participant before enrolment, and all procedures were conducted in strict accordance with the ethical regulations of the College of Medicine, University of Al-Qadisiyah and the Declaration of Helsinki.

### 2.1 Study Design and Participants

A case-control study was conducted between September 15, 2025, and April 15, 2026. A total of 120 participants were enrolled across three groups: 60 apparently healthy individuals serving as controls, 30 patients with T2DM diagnosed by a specialist at the Diabetes Center of Sadr Medical City and Al-Hakim General Hospital according to ADA 2024 diagnostic criteria, and 30 patients with DN diagnosed by a nephrologist at the Kidney Center of Sadr Medical City on the basis of clinical findings and laboratory measurements. All biochemical analyses were carried out at the Clinical Chemistry Research Laboratory, College of Medicine, University of Al-Qadisiyah, and at the Clinical Chemistry Laboratory of Al-Sadr Teaching Hospital. BMI was calculated as weight in kilograms divided by the square of height in meters.

### 2.2 Inclusion and Exclusion Criteria

Patients of both sexes aged between 30 and 70 years with a clinically confirmed diagnosis of T2DM or DN were eligible (UACR > 30 mg/g, eGFR < 60 mL/min) Healthy controls were required to be free of cardiovascular disease, diabetes, hypertension, renal disease, endocrine disorders, and any acute or chronic illness at the time of enrolment, confirmed by a structured health questionnaire. Controls were matched to the patient groups in approximate age range. Participants were excluded if they had type 1 diabetes mellitus, a history of eye, kidney, or nerve infections, liver disease, a systemic autoimmune condition, any malignancy, current pregnancy, or primary hypertension.

### 2.3 Blood Sampling

A 5 mL venous blood sample was collected from each participant. Three mL were transferred to serum-separating gel tubes and allowed to coagulate for one hour at room temperature, then centrifuged at 3,000×g for 10 minutes; 2 mL were collected in EDTA-anticoagulated tubes for flow cytometric analysis. The resulting serum was divided into aliquots and stored at −20°C until analysis.

### 2.4 Biochemical Assays

Fasting blood glucose (FBG) was measured by the glucose oxidase–peroxidase colorimetric method at 500 nm (Glucose Kit, Cat. No. 34018, Biosystem, Spain). HbA1c was determined by a fully automated boronate affinity assay using the Affinion® analyser (Alere, USA). Serum urea was determined by the urease–glutamate dehydrogenase method at 600 nm (Urea/BUN Kit, Cat. No. 35060, Biosystem, Spain). Serum creatinine was quantified by the Jaffe alkaline picrate kinetic reaction at 510 nm (Creatinine Kit, Cat. No. 37647, Biosystem, Spain). Total cholesterol and triglycerides were measured by enzymatic colorimetric methods at 500 nm (Cat. No. 37941 and 35204 respectively, Biosystem, Spain). All spectrophotometric measurements were performed on a Shimadzu spectrophotometer (Japan).

### 2.5 Measurement of Serum UMOD and NGAL by ELISA

Serum UMOD and NGAL concentrations were quantified by a sandwich ELISA using commercially validated kits from BT LAB, China: Human Uromodulin ELISA Kit (Cat. No. 4743Hu) and Human Neutrophil Gelatinase Associated Lipocalin ELISA Kit (Cat. No. E1719Hu), performed in strict accordance with the manufacturer's instructions. Absorbance was read at 450 nm using a microplate reader (Karl Kolb, Germany). A five-point serial dilution standard curve was constructed for each biomarker and unknown sample concentrations were calculated by regression analysis.

### 2.6 Flow Cytometric Analysis of CD4+ T Cells

Peripheral blood CD4+ T cell percentages were determined by flow cytometry as follows: Briefly, 100 µL of fresh EDTA-anticoagulated whole blood was incubated with anti-human CD4-FITC monoclonal antibody (BD Biosciences, USA) for 20 minutes at room temperature in the dark, followed by red blood cell lysis using BD FACS Lysing Solution. Samples were acquired on a flow cytometer and a minimum of 10,000 events were collected per sample. Data were analysed using FlowJo software (version 10.0; BD Biosciences, USA) with gating on the

lymphocyte population. Results are expressed as percentage of CD4+ cells within total lymphocytes. the flow cytometry data underlying the CD4+ T cell percentages organised as a two-panel table: Panel A shows the scatter plots (SSC-H vs. FSC-H) used to define the lymphocyte gate (P1%), and Panel B shows the corresponding FITC fluorescence intensity histograms (I1%) confirming CD4+ surface expression, for each of the three study groups.

### 2.7 Statistical Analysis

Data were described and analysed using GraphPad Prism version 11.0 (GraphPad Software, San Diego, CA, USA). Sample size was calculated based on expected effect size from pilot data (power 80%, $\alpha$=0.05). Continuous variables are expressed as mean ± standard deviation (SD), while for non-normal data, use median (IQR) or log transform. Data normality was assessed by the Shapiro–Wilk test. Differences among the three groups were evaluated by one-way analysis of variance (ANOVA), Tukey's honestly significant difference (HSD) post hoc test was applied to perform all pairwise comparisons between groups. A *P*-value of ≤0.05 was considered statistically significant throughout. Diagnostic performance was assessed by ROC curve analysis); AUC and 95% CIs are reported.

## 3- RESULTS AND DISCUSSION

### 3.1 Demographic and Baseline Characteristics

Table 1 presents the demographic and baseline biochemical characteristics of the three study groups. Age, sex distribution, and BMI were comparable across all groups, with no statistically significant differences ($P$ = 0.130, 0.872, and 0.764, respectively), confirming adequate group matching. In contrast, FBG, HbA1c, serum urea, and creatinine were highly significantly different across groups ($P$ < 0.0001 for all), validating the clinical distinction between controls, T2DM patients, and DN patients.

**Table (1): Demographic and Baseline Biochemical Characteristics of the Three Study Groups**

| **Parameter** | **Control (n=60) Mean ± SD** | **T2DM (n=30) Mean ± SD** | **DN (n=30) Mean ± SD** | ***P*-value** |
|---|---|---|---|---|
| **Age (years)** | 61.60 ± 10.66 | 63.92 ± 8.58 | 65.00 ± 11.58 | 0.1300 |
| **Sex (M/F)** | 21/39 | 10/20 | 11/19 | 0.8720 |
| **BMI (kg/m²)** | 27.90 ± 5.09 | 27.22 ± 2.99 | 26.99 ± 4.01 | 0.7640 |
| **FBG (mg/dL)** | 94.99 ± 16.25 | 308.80 ± 81.78 | 350.00 ± 86.30 | <0.0001 |
| **HbA1c (%)** | 5.20 ± 0.40 | 9.89 ± 2.09 | 8.10 ± 1.51 | <0.0001 |
| **Serum Urea (mg/dL)** | 32.17 ± 5.76 | 33.70 ± 7.98 | 68.00 ± 29.66 | <0.0001 |
| **Creatinine (mg/dL)** | 0.83 ± 0.53 | 0.74 ± 0.21 | 2.00 ± 0.73 | <0.0001 |
| **eGFR** | 109.38 ±10.69 | 108.24±17.8 | 36.00 ±12.19 | <0.0001 |
| **Systolic pressure (mmHg)** | 12.22±0.70 | 13.32±0.85 | 13±1.10 | 0.77 |
| **Diastolic pressure (mmHg)** | 7.46 ±0.53 | 8.44±2.10 | 7.46±1.43 | 0/85 |

**Values are mean ± SD. P-value from one-way ANOVA with Tukey post-hoc test. FBG: Fasting blood glucose; HbA1c: Glycated haemoglobin; BMI: Body mass index**

### 3.2 Serum Uromodulin (UMOD) Concentrations

Serum UMOD concentrations differed significantly across the three groups, with a clear and progressive stage-dependent increase from healthy controls through T2DM to DN patients (Figure 1). Mean serum UMOD was (53.02 ± 11.79 ng/mL) in the control group, rising to (89.81 ± 24.82 ng/mL) in T2DM patients, and reaching (105.97 ± 26.36 ng/mL) in DN patients. One-way ANOVA confirmed a highly significant overall group effect ($P$ < 0.001). and that DN patients had significantly higher UMOD than T2DM patients ($P$ = 0.028), indicating that the elevation in serum UMOD tracks disease progression in a graded, stage-dependent manner.

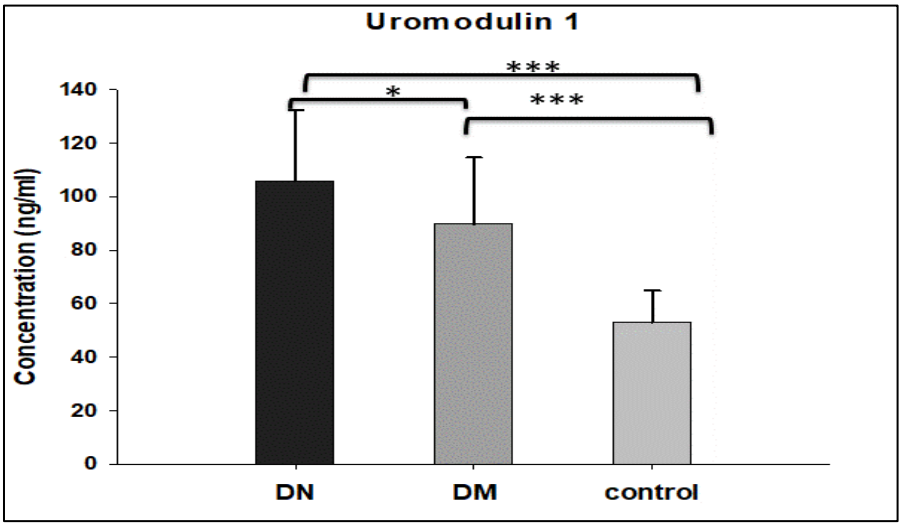


**Fig (1): Serum UMOD concentrations (ng/mL) in healthy controls, T2DM patients, and DN patients. Bars represent mean ± SD. *$P$ < 0.05; ***$P$ < 0.001**

### 3.3 Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) Concentrations

Serum NGAL concentrations showed a marked statistically significant progressive elevation across the three study groups (Figure 2). Mean serum NGAL was (80.51±24.03 ng/mL) in healthy controls, increasing substantially to (240. 04 ± 42.68 ng/mL) in T2DM patients, and reaching (290.19±52.17 ng/mL) in DN patients. One-way ANOVA demonstrated a highly significant overall group effect ($P$ < 0.001). Tukey's HSD post-hoc test confirmed highly significant differences between all three group pairs, with controls versus T2DM, controls versus DN, and T2DM versus DN all reaching ($P$ < 0.001, Table 3). These findings indicate that serum NGAL rises substantially even at the uncomplicated T2DM stage and continues to escalate as nephropathy becomes established.

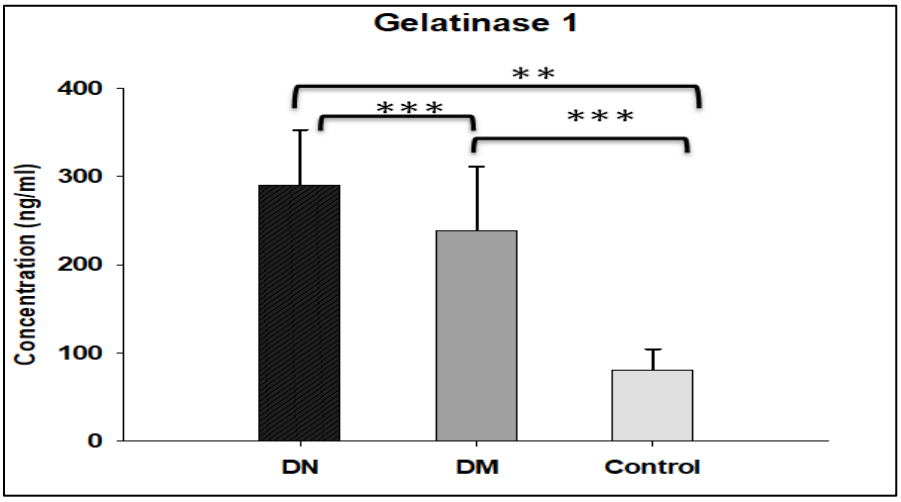


**Fig (2): Serum NGAL concentrations (ng/mL) in healthy controls, T2DM patients, and DN patients. Data are presented as mean ± SD. **$P$ <0.01 , ***$P$ < 0.001**

### 3.4 CD4+ T Cell Percentages by Flow Cytometry

Flow cytometric analysis of peripheral blood CD4+ T cell percentages revealed significant differences among the three study groups. Mean CD4+ T cell percentage was increased to (59.21 ± 5.22) in DN patients and (41.50 ± 5.37) in T2DM patients than in healthy control (32.56 ± 4.40) (Figure 3 and 4). One-way ANOVA demonstrated that T2DM patients and DN had significantly higher CD4+ T cell percentages compared with healthy controls ($P < 0.001$). Also, significant increase was indicated between patient groups compared to control ($P < 0.001$. These findings indicate a selective activation of circulating CD4+ T helper cells in the patients with DN.

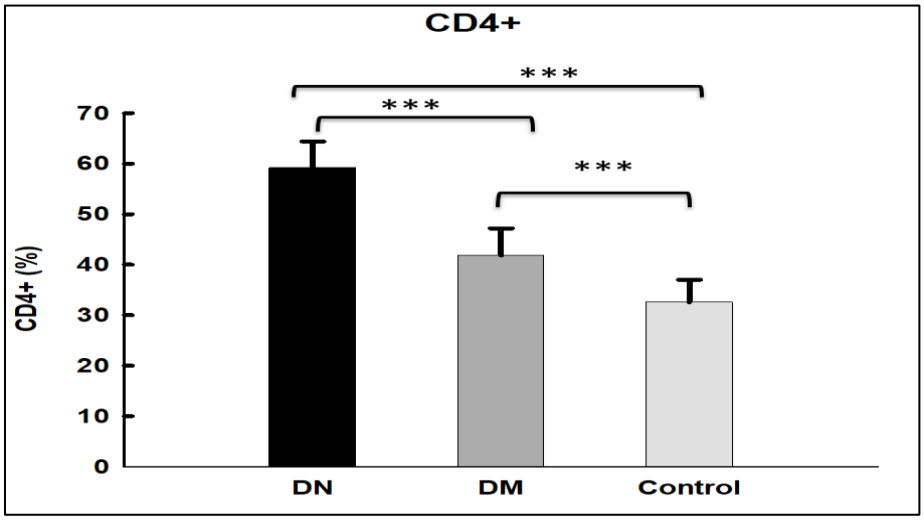


**Fig (3): CD4+ percentage in healthy controls, T2DM patients, and DN patients. Data as represent mean ± SD**

***$P < 0.0001$**

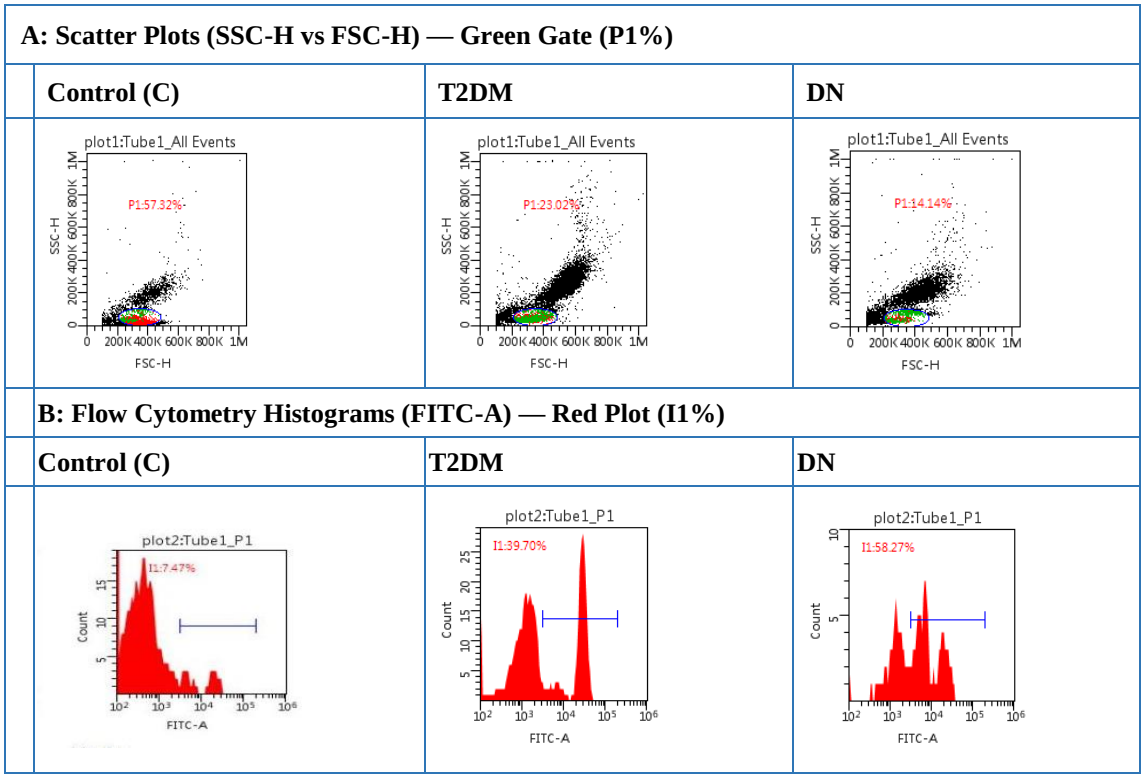


**Fig (4): A flowcytometry diagram. (A)Scatter Plots (SSC-H vs FSC-H) — Green Gate and B: Flow Cytometry Histograms (FITC-A), Red Plot (I1%), and (P1%) for patient groups and control**

### 3.5 Diagnostic Performance: ROC Curve Analysis

ROC curve analysis was performed for each biomarker to assess its ability to discriminate T2DM and DN patients from healthy controls (Table 2, Figures 5). Serum UMOD demonstrated excellent discriminatory performance: (AUC = 0.948 ,95% CI 0.908–0.988, *P* < 0.001) for T2DM and (AUC = 0.992, 95% CI 0.980–1.004, *P* < 0.001) for DN. Serum NGAL showed near-perfect discrimination: AUC = 0.995 (95% CI 0.987–1.003) for T2DM and (AUC = 1.000, 95% CI 1.000–1.000) for DN (P < 0.001). CD4+ T cell percentage showed good discrimination for T2DM (AUC = 0.802, 95% CI 0.704–0.901, *P* < 0.001) and excellent discrimination for DN (AUC = 0.999, 95% CI 0.998–1.001, *P* < 0.001).

**Table (2): ROC Curve Analysis for UMOD, NGAL, and CD4+ T Cells**

| Biomarker | Comparison | AUC | 95% CI | Cut off | Sensitivity% | Specifity% | P-value |
|---|---|---|---|---|---|---|---|
| **UMOD (ng/mL)** | T2DM | 0.948 | 0.908–0.988 | <63.00 | 84.6 | 80.4 | <0.001 |
| | DN | 0.992 | 0.980–1.004 | <72.5 | 77.9 | 71.3 | <0.001 |
| **NGAL (ng/mL)** | T2DM | 0.995 | 0.987–1.003 | <98.21 | 82.1 | 76.8 | <0.001 |
| | DN | 1.000 | 1.000–1.000 | <114.56 | 100 | 100 | <0.001 |
| **CD4+ (%)** | T2DM | 0.802 | 0.704–0.901 | <39.35 | 70.5 | 64.3 | <0.001 |
| | DN | 0.999 | 0.998–1.001 | <42.69 | 80.1 | 66.7 | <0.001 |

**AUC: area under the curve; CI: confidence interval. All comparison vs. healthy controls as reference**
**AUC > 0.9 = excellent diagnostic performance**

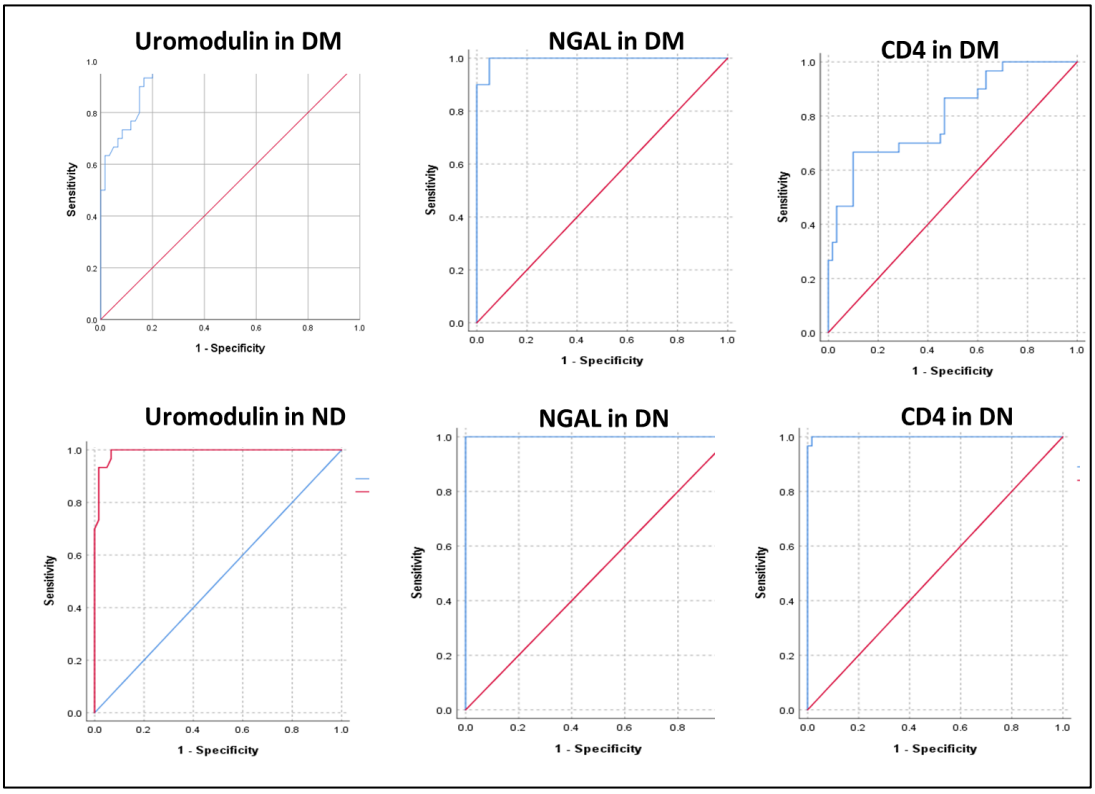


**Fig (5): ROC Curves for Biomarkers in T2DM and DN**

This study simultaneously evaluated serum UMOD, serum NGAL, and peripheral blood CD4+ T cell percentages across three well-defined groups, healthy controls, T2DM patients, and DN patients, All three markers demonstrated significant, stage-dependent changes that tracked disease progression from uncomplicated diabetes through to established nephropathy, providing evidence that tubular and immunological dysfunction are detectable at a molecular level. Serum UMOD rose progressively in T2DM patients and DN patients, with statistically significant differences ($P < 0.001$ from control with AUC values of 0.948 and 0.992 for T2DM and DN respectively, indicating excellent diagnostic performance consistent with published uromodulin data [٩].This graded elevation is consistent with a compensatory phase in which hyperglycaemia-driven tubular hypertrophy and increased basolateral UMOD secretion transiently raise circulating UMOD before irreversible cell loss supervenes in established nephropathy. The progressive rise from T2DM to DN suggests that the thick ascending limb remains metabolically active but increasingly stressed across these stages, and that serum UMOD tracks this evolving burden with stage-specific fidelity [16]. Uromodulin is synthesised exclusively by the epithelial cells of the thick ascending limb and is released constitutively into the circulation at low levels, where its concentration directly reflects the size and functional state of the viable tubular cell mass [17]. Under chronic hyperglycaemia, this segment is exposed to converging injurious stimuli: mitochondrial dysfunction driven by glucose toxicity and advanced glycation end-product accumulation, and the resulting oxidative stress collectively impose a chronic metabolic load on thick ascending limb cells [18]. Under these conditions, compensatory tubular hypertrophy and upregulated basolateral UMOD secretion transiently increase circulating UMOD, producing the stage-dependent rise observed in this cohort before irreversible cell loss eventually reverses this trend in advanced nephropathy [19].

The finding that serum UMOD was already significantly elevated at the T2DM stage aligns with prospective cohort evidence that UMOD concentrations begin to diverge from normal values before albuminuria or eGFR decline reach diagnostic thresholds [20], reflecting early compensatory changes in thick ascending limb activity [21]. A previous study confirmed in a large prospective cohort that UMOD independently predicted eGFR decline and all-cause mortality in T2DM patients, underscoring its prognostic as well as diagnostic relevance, Because UMOD is produced by only one nephron segment, its circulating levels are not confounded by systemic inflammation, infection, or liver disease, a property that makes it particularly valuable in the multi-morbid T2DM population [22].
Serum NGAL showed the most dramatic change across groups, rising in T2DM and DN patients, with a highly significant ($P < 0.001$). The magnitude of the NGAL elevation more than 2.7-fold from controls to T2DM and more than a 3.3-fold increase from controls to DN, reflects the intensity of ongoing tubular cell stress rather than simply cumulative cell loss, Serum NGAL was near-perfect in both comparisons (AUC = 0.995 for T2DM and 1.000 for DN), in line with its established role as an acute tubular injury marker [23]. The AUC for $CD4^+$ T cells was (0.802) for T2DM and approached perfection for DN (0.999), indicating that $CD4^+$ T cell suppression is a more consistent feature in established nephropathy than in uncomplicated T2DM. NGAL is a 25-kDa lipocalin that is constitutively expressed at low levels in tubular epithelial cells but is dramatically upregulated, by 10 to 100-fold, within hours of any significant tubular stress or injury. In the diabetic kidney, the combination of glucose-derived reactive oxygen species and chronic low-grade inflammatory signaling creates a self-reinforcing loop that sustains chronic NGAL upregulation even at the early T2DM stage [24]. The substantially higher NGAL values observed in DN patients compared with T2DM patients indicate that tubular stress escalates further as nephropathy becomes established and both the structural damage and the systemic inflammatory burden increase. Kapoula *et al.* (2019) confirmed in a meta-analysis that NGAL rises significantly in T2DM relative to healthy individuals and predicts DN development with clinically meaningful accuracy [25].

Flow cytometric analysis revealed that $CD4^+$ T-cell percentages were significantly higher in patients with T2DM and DN than in healthy controls ($P < 0.001$). $CD4^+$ T helper cells are central orchestrators of adaptive immunity and inflammatory tissue remodeling in diabetic kidney disease. In T2DM, chronic hyperglycaemia and the resulting mitochondrial dysfunction create a paradoxical immune state in which persistent low-grade inflammation coexists with impaired $CD4^+$ T-cell proliferative capacity. Advanced glycation end-products activate RAGE signaling and downstream NF-κB pathways that suppress T-cell receptor-mediated activation, while the expansion of myeloid-derived suppressor cells and regulatory T cells within the metabolically stressed environment further constrains the $CD4^+$ effector compartment [24]. Furthermore, mitochondrial dysfunction in T2DM impairs the oxidative phosphorylation required for T-cell activation and clonal expansion, thereby compromising $CD4^+$ T-cell function despite the presence of systemic inflammation [26]. The observed increase in $CD4^+$ T-cell percentages in DN is biologically plausible and is consistent with immune reorganization driven by the intensified inflammatory milieu accompanying established nephropathy. As tubular injury progresses and damage-associated molecular patterns are released from injured renal cells, chronic antigenic stimulation promotes the recruitment and expansion of $CD4^+$ T cells, partially overcoming the immune suppression observed during the earlier stages of T2DM [27,28]. However, it

is important to recognize that the increased percentage of circulating $CD4^+$ T cells represents a relative distribution within the lymphocyte population rather than a direct measure of their functional activation or effector capacity. Functional characteristics such as cytokine production, proliferative responses, metabolic fitness, and expression of activation markers (e.g., CD69, CD25, or HLA-DR) cannot be determined from percentage measurements alone. Moreover, interpretation of $CD4^+$ T-cell percentages should be made with caution because relative percentages may be influenced by changes in other leukocyte subsets, total lymphocyte counts, or alterations in immune cell trafficking during chronic inflammation. Consequently, an elevated $CD4^+$ T-cell percentage does not necessarily indicate enhanced immune competence or pathogenic activity. Comprehensive assessment of $CD4^+$ T-cell involvement in diabetic nephropathy would require integration of absolute cell counts with functional assays and phenotypic characterization of T-cell subsets. Nevertheless, the observed changes in $CD4^+$ T-cell percentages provide supportive evidence of immune dysregulation during diabetic renal progression and complement the multi-biomarker profile identified in this study, consistent with the broader evidence that innate and adaptive immune responses evolve in a coordinated manner as nephropathy becomes established [29]. From a clinical perspective, the stage-dependent rise in tubular and inflammatory biomarkers observed here is consistent with the renoprotective benefits reported for SGLT2 inhibitors in patients with T2DM and CKD, as well as for non-steroidal mineralocorticoid receptor antagonists in T2D-related kidney disease, suggesting that serial measurement of UMOD and NGAL could help identify patients most likely to benefit from early initiation of these therapies [30].

## 4- CONCLUSION

Serum UMOD, NGAL, and CD4+ T-cell percentages showed significant alterations in patients with T2DM and DN and demonstrated good-to-excellent diagnostic performance. Among these biomarkers, NGAL demonstrated the highest diagnostic accuracy, suggesting that it may be a particularly useful marker for the early detection and assessment of diabetic nephropathy. These findings support the potential utility of UMOD, NGAL, and CD4+ T-cell percentages as noninvasive biomarkers for disease monitoring and risk stratification. This study was limited by its single-center, cross-sectional design and relatively small sample size, which may limit the generalizability of the findings. In addition, biomarker measurements were performed at a single time point, and $CD4^+$ T-cell subsets and functional immune analyses were not evaluated. Therefore, larger prospective multicenter studies are needed to validate these findings.

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