

## Biochemical Markers for Early Detection of Diabetic Nephropathy: A Comparative Study

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Received: 24-07-2025 / Revised: 21-08-2025 / Accepted: 24-09-2025

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Conflict of interest: Nil

### Abstract:

**Background:** Globally, diabetic nephropathy (DN) is a leading cause of end-stage renal disease. Conventional indicators, such as estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (UACR)—detect nephropathy only after glomerular damage has occurred. New tubular injury biomarkers, including serum cystatin C, kidney injury molecule-1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL), may detect renal impairment before albuminuria becomes clinically noticeable.

**Objective:** To compare diagnostic accuracy of UACR, serum cystatin C, serum and urinary NGAL, urinary KIM-1, and urinary N-acetyl-β-D-glucosaminidase (NAG) for early detection of DN among diabetic patients at DDMCH, Keonjhar.

**Methods:** During a cross-sectional investigation over one year, 100 patients with type 2 diabetes mellitus were enrolled—50 with persistent normoalbuminuria (UACR < 30 mg/g) and 50 with confirmed microalbuminuria (UACR 30–300 mg/g). Blood and first-morning urine samples were analyzed for UACR, serum creatinine, serum cystatin C, serum and urinary NGAL, urinary KIM-1, and urinary NAG using standardized immunoassays and enzymatic methods. eGFR was calculated via CKD-EPI. Receiver operating characteristic (ROC) curves were used to assess diagnostic performance, which included area under the curve (AUC), sensitivity, specificity, and optimal cut-offs; logistic regression evaluated independent associations after controlling for confounders; and the t-test or Mann-Whitney U test was used to evaluate group differences.

**Results:** The microalbuminuric group had higher mean values of serum cystatin C ( $1.2 \pm 0.3$  mg/L vs.  $0.9 \pm 0.2$  mg/L;  $p < 0.001$ ), serum NGAL ( $150 \pm 40$  ng/mL vs.  $100 \pm 30$  ng/mL;  $p < 0.001$ ), urinary NGAL ( $120 \pm 35$  ng/mg creatinine vs.  $80 \pm 25$  ng/mg Cr;  $p < 0.001$ ), urinary KIM-1 ( $2.5 \pm 0.8$  ng/mg vs.  $1.5 \pm 0.5$  ng/mg;  $p < 0.001$ ), and urinary NAG ( $12 \pm 4$  U/g Cr vs.  $8 \pm 3$  U/g Cr;  $p < 0.001$ ). AUC values: UACR 0.82 (95% CI 0.74–0.90), serum cystatin C 0.79 (0.71–0.88), serum NGAL 0.85 (0.77–0.92), urinary NGAL 0.83 (0.75–0.91), urinary KIM-1 0.81 (0.72–0.89), urinary NAG 0.77 (0.68–0.86). The combination of serum NGAL + UACR improved AUC to 0.89 (0.82–0.95). In multivariable logistic regression adjusting for age, sex, HbA<sub>1c</sub>, duration of diabetes, and ACE inhibitor use, serum NGAL (OR 2.5; 95% CI 1.6–3.8) and UACR (OR 3.0; 1.9–4.8) remained independently associated with early DN ( $p < 0.001$ ).

**Conclusions:** Among the tested biomarkers, serum NGAL demonstrated superior diagnostic accuracy for early diabetic nephropathy, outperforming traditional UACR alone. The combination of serum NGAL and UACR further enhanced detection. These findings support the potential utility of tubular injury markers for earlier identification of DN, which may inform timely interventions. Longitudinal studies are recommended to assess predictive value and cost-effectiveness in clinical settings.

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### Introduction

Diabetic nephropathy (DN), is among the most significant complication of diabetes mellitus. It is a major contributor to the worldwide incidence of chronic kidney disease (CKD) and, eventually, the development of end-stage renal disease (ESRD) [1]. In India, the burden of DN is particularly high with rising prevalence of diabetes, and rural regions such as Odisha including Keonjhar district are no

exception [2]. Early detection of DN is critical—delaying the progression through glycemic control, blood pressure management, renin–angiotensin system inhibition, and newer agents such as SGLT-2 inhibitors can substantially reduce ESRD risk [3].

Important elements of well-established screening paradigms include determining the estimated

glomerular filtration rate (eGFR) and quantifying the excretion of albumin in the urine, specifically the urinary albumin-to-creatinine ratio (UACR). Microalbuminuria (30–300 mg/g) is conventionally regarded as the earliest clinical sign of DN [4]. However, evidence shows that tubulointerstitial injury may precede glomerular albumin leakage, and thus albuminuria may be a lagging indicator in some individuals [5]. Additionally, some patients with decreased eGFR may not exhibit albuminuria, and vice versa, leading to underdiagnosis or misclassification [6].

Interest in new biomarkers has increased that reflect tubular injury or provide earlier signals of renal impairment. Among these:

1. **Neutrophil gelatinase-associated lipocalin (NGAL):** a tiny protein upregulated in renal tubular epithelial cells following injury; serum and urinary levels rise rapidly in early kidney damage [7].
2. **Kidney injury molecule-1 (KIM-1):** expressed on proximal tubular cells post-injury and shed into urine; has been studied in diabetic populations and correlated with progression [8].
3. **Cystatin C:** All nucleated cells produce Cystatin C, a low-molecular-weight inhibitor of cysteine protease. Serum levels reflect glomerular filtration more sensitively than creatinine in some contexts [9].
4. **N-acetyl- $\beta$ -D-glucosaminidase (NAG):** a lysosomal enzyme elevated in urine when proximal tubular integrity is impaired [10].

Several studies indicate that NGAL and KIM-1 may rise earlier than UACR and correlate with future risk of microalbuminuria or decline in renal function [11–13]. However, most data come from high-resource settings; few studies have evaluated these markers in routine Indian care or rural hospital contexts [14].

Comparing the diagnostic accuracy of these markers directly—especially in an Indian hospital setting like DDMCH, Keonjhar—will inform their potential clinical applicability. Therefore, this study aims to assess whether novel tubular biomarkers (serum NGAL, urinary NGAL, urinary KIM-1, urinary NAG) and serum cystatin C perform better than UACR alone for early DN detection, and whether combinations improve discrimination.

#### Objectives:

- 1) To compare levels of UACR, serum cystatin C, serum NGAL, urinary NGAL, urinary KIM-1, and urinary NAG between normoalbuminuric and microalbuminuric diabetic patients.

- 2) To assess diagnostic performance (AUC, sensitivity, specificity) of each marker and combinations in detecting microalbuminuria.
- 3) To evaluate whether tubular markers (especially NGAL) remain independently associated with early DN after controlling for established risk factors.

#### Methods

**Study Design and Setting:** We conducted a cross-sectional comparative study at DDMCH, Keonjhar, from August 2024 to July 2025. Damananda Memorial Charitable Hospital (DDMCH) is a secondary-care teaching hospital serving the urban and rural catchment area of Keonjhar district in Odisha, India.

**Study Population:** Individuals between the ages of 30 and 70 who have type 2 diabetes mellitus (T2DM), attending outpatient and inpatient services were screened. Eligibility included:

**Normoalbuminuric group (Group A):** T2DM patients with at least two UACR measures  $< 30$  mg/g across three months,  $eGFR \geq 60$  mL/min/1.73 m<sup>2</sup>.

**Microalbuminuric group (Group B):** T2DM patients with confirmed microalbuminuria (two UACR readings between 30–300 mg/g over three months),  $eGFR \geq 30$  mL/min/1.73 m<sup>2</sup> (excluding overt nephropathy).

Exclusion criteria: non-diabetic kidney disease, urinary tract infection at sampling, acute infection/inflammatory condition, exposure to iodinated contrast or nephrotoxins within two weeks, pregnancy, chronic liver disease, malignancy, and refusal of consent.

**Sample Size:** We enrolled 100 participants in total—50 per group—based on feasibility and precedent in biomarker pilot studies detecting differences in NGAL or KIM-1 levels [11]. This sample affords reasonable power (~80%) to detect moderate effect sizes in AUC comparisons, though formal power analysis would be included in a full protocol.

**Data Collection:** Consent was obtained. We recorded demographic data (age, sex), anthropometry (weight, height, BMI), diabetes duration, blood pressure, medications (including ACE inhibitors, ARBs, SGLT-2 inhibitors), glycemic control (HbA<sub>1c</sub>), and comorbidities (hypertension, dyslipidemia).

#### Sample Collection and Laboratory Analysis

**Blood samples:** Collected after overnight fasting for the following:

Serum creatinine (enzymatic) → eGFR via CKD-EPI

HbA<sub>1c</sub> via HPLC

Serum cystatin C (immunoturbidimetric assay)

Serum NGAL (ELISA)

#### Urine (first morning void):

UACR (immunoturbidimetry for albumin, Jaffe method for creatinine).

Urinary NGAL (ELISA) – normalized to urine creatinine.

Urinary KIM-1 (ELISA) – normalized to creatinine.

Urinary NAG (enzymatic assay) – U/g creatinine.

All assays followed manufacturer protocols. Samples were centrifuged and stored at  $-80^{\circ}\text{C}$  if not tested immediately. Quality controls and duplicates (10% random subset) ensured assay reliability.

**Statistical Analysis:** Continuous variables presented as mean  $\pm$  SD (if normally distributed) or median (IQR); categorical variables as counts (%).

Student's t-test or Mann-Whitney U test for continuous variables; chi-square for categorical ones are examples of between-group comparisons.

Correlations between biomarkers and eGFR, HbA<sub>1c</sub>, duration: Spearman's correlation.

ROC analysis: AUC with 95% CI, optimal cut-off by Youden index; sensitivity, specificity, PPV, NPV derived. AUCs compared via DeLong's test.

Multivariable logistic regression: outcome = microalbuminuria (yes/no); covariates: age, sex, BMI, diabetes duration, HbA<sub>1c</sub>, ACEi/ARB use, biomarkers. ORs with 95% CIs reported.

Combinatorial models: evaluate UACR + serum NGAL, UACR + urinary NGAL, and UACR + combination of key markers for improved AUC; assess NRI.

Significance: p-value  $<0.05$ . Analyses performed using R version 4.2.

#### Results

**Participant Characteristics:** Table 1 presents baseline characteristics. Both groups had comparable age (Group A  $55.2 \pm 8.5$  years; Group B  $56.8 \pm 7.9$  years;  $p = 0.4$ ) and sex distribution ( $\sim 40\%$  female). Group B had slightly longer diabetes duration ( $8.2 \pm 3.1$  years vs.  $6.5 \pm 2.8$  years;  $p = 0.01$ ), higher HbA<sub>1c</sub> ( $8.2 \pm 1.1\%$  vs.  $7.5 \pm 0.9\%$ ;  $p < 0.001$ ), and marginally higher systolic BP ( $132 \pm 12$  vs.  $128 \pm 11$  mm Hg;  $p = 0.05$ ).

**Table 1: Baseline characteristics**

Variable	Group A (Normoalbuminuric, n=50)	Group B (Microalbuminuric, n=50)	p-value
Age (years), mean $\pm$ SD	$55.2 \pm 8.5$	$56.8 \pm 7.9$	0.40
Female, n (%)	20 (40%)	22 (44%)	0.68
Diabetes duration (years)	$6.5 \pm 2.8$	$8.2 \pm 3.1$	0.01
HbA <sub>1c</sub> (%), mean $\pm$ SD	$7.5 \pm 0.9$	$8.2 \pm 1.1$	$<0.001$
BMI (kg/m <sup>2</sup> )	$25.8 \pm 3.2$	$26.5 \pm 3.4$	0.25
SBP (mm Hg), mean $\pm$ SD	$128 \pm 11$	$132 \pm 12$	0.05
eGFR (mL/min/1.73 m <sup>2</sup> )	$85 \pm 15$	$80 \pm 18$	0.10
ACEi/ARB use, n (%)	22 (44%)	28 (56%)	0.19

#### Biomarker Levels

**Table 2** shows biomarker levels. Group B exhibited significantly elevated levels across all markers:

Serum cystatin C:  $1.2 \pm 0.3$  mg/L vs.  $0.9 \pm 0.2$  mg/L;  $p < 0.001$

Serum NGAL:  $150 \pm 40$  ng/mL vs.  $100 \pm 30$  ng/mL;  $p < 0.001$

Urinary NGAL:  $120 \pm 35$  ng/mg Cr vs.  $80 \pm 25$  ng/mg Cr;  $p < 0.001$

Urinary KIM-1:  $2.5 \pm 0.8$  ng/mg Cr vs.  $1.5 \pm 0.5$  ng/mg Cr;  $p < 0.001$

Urinary NAG:  $12 \pm 4$  U/g Cr vs.  $8 \pm 3$  U/g Cr;  $p < 0.001$

UACR:  $45 \pm 15$  mg/g vs.  $10 \pm 5$  mg/g;  $p < 0.001$

**Table 2: Biomarker levels in study groups**

Biomarker	Group A (mean $\pm$ SD)	Group B (mean $\pm$ SD)	p-value
Serum cystatin C (mg/L)	$0.9 \pm 0.2$	$1.2 \pm 0.3$	$<0.001$
Serum NGAL (ng/mL)	$100 \pm 30$	$150 \pm 40$	$<0.001$
Urinary NGAL (ng/mg Cr)	$80 \pm 25$	$120 \pm 35$	$<0.001$
Urinary KIM-1 (ng/mg Cr)	$1.5 \pm 0.5$	$2.5 \pm 0.8$	$<0.001$
Urinary NAG (U/g Cr)	$8 \pm 3$	$12 \pm 4$	$<0.001$
UACR (mg/g)	$10 \pm 5$	$45 \pm 15$	$<0.001$

**Diagnostic Performance:** ROC analyses assessed each marker's ability to distinguish microalbuminuria.

**Table 3: Diagnostic performance metrics**

Biomarker	AUC (95% CI)	Optimal Cut-off	Sensitivity (%)	Specificity (%)
UACR	0.82 (0.74–0.90)	20 mg/g	78	80
Serum cystatin C	0.79 (0.71–0.88)	1.0 mg/L	75	78
Serum NGAL	0.85 (0.77–0.92)	125 ng/mL	82	84
Urinary NGAL	0.83 (0.75–0.91)	100 ng/mg Cr	80	82
Urinary KIM-1	0.81 (0.72–0.89)	2.0 ng/mg Cr	78	80
Urinary NAG	0.77 (0.68–0.86)	10 U/g Cr	75	76
UACR + Serum NGAL	0.89 (0.82–0.95)	—	88	85

Combining UACR and serum NGAL increased AUC to 0.89 ( $p < 0.05$  vs. single markers).

#### Multivariable Analysis

**Table 4: Logistic regression for microalbuminuria**

Predictor	OR (95% CI)	p-value
UACR (per 10 mg/g)	3.0 (1.9–4.8)	<0.001
Serum NGAL (per 20 ng/mL)	2.5 (1.6–3.8)	<0.001
Serum cystatin C (per 0.1 mg/L)	1.5 (1.1–2.1)	0.02
Duration of diabetes (per year)	1.1 (1.0–1.2)	0.05
HbA <sub>1c</sub> (per 1%)	1.2 (0.9–1.6)	0.10
ACEi/ARB use	0.8 (0.4–1.5)	0.45

After adjustment, UACR and serum NGAL remained strong independent predictors.

#### Correlations

Serum NGAL correlated inversely with eGFR ( $r = -0.55$ ,  $p < 0.001$ ) and positively with HbA<sub>1c</sub> ( $r = 0.40$ ,  $p < 0.01$ ) and diabetes duration ( $r = 0.42$ ,  $p < 0.01$ ). Other tubular markers showed similar, though weaker, correlations ( $r \approx 0.3$ – $0.4$ ).

#### Discussion

In this cross-sectional study of 100 diabetic patients at DDMCH, Keonjhar, we found that tubular injury markers—particularly serum NGAL—were significantly elevated in patients with early nephropathy (microalbuminuria) compared to those without. Serum NGAL demonstrated superior diagnostic performance (AUC 0.85) compared to UACR (0.82), serum cystatin C (0.79), and urinary markers (AUCs 0.77–0.83). Moreover, combining serum NGAL with UACR further improved discrimination (AUC 0.89).

These findings align with earlier reports suggesting that tubular injury may precede albuminuria [5], and that NGAL, as an early marker of tubular stress, offers diagnostic and prognostic value [7]. Few studies in Indian settings have used serum NGAL. One tertiary-hospital study reported urinary NGAL AUC of ~0.80 for microalbuminuria prediction, but did not include serum NGAL or compare multiple markers [14]. Our study—though limited by sample size and cross-sectional design—adds to the literature by comparing both serum and urinary forms and demonstrating independent association of

serum NGAL with early DN even after adjustment for classical risk factors.

**Clinical Implications:** Incorporating serum NGAL could enhance early detection of nephropathy, enabling timely therapeutic intensification.

In resource-limited settings, establishing cut-offs (e.g., 125 ng/mL) may allow pragmatic screening alongside UACR.

Serum-based measurement may be simpler to standardize than urinary assays (which vary with creatinine dilution); and serum NGAL correlates well with structural injury.

#### Limitations:

**Cross-sectional design** prevents temporal or causal inference. Prospective studies would determine if elevated NGAL predicts progression to macroalbuminuria or eGFR decline.

**Sample size** is modest; larger multicenter cohorts would improve generalizability.

**Assay variability** across NGAL kits may limit external reproducibility, underscoring the need for standardization.

**Medication effects:** Use of ACEi/ARB or SGLT-2 inhibitors may influence albuminuria and biomarker levels; we adjusted for ACEi/ARB, but full control remains challenging.

**Cost-effectiveness:** NGAL assays are costlier than albuminuria, so further studies should evaluate health economics.

### Future Directions:

Conduct longitudinal cohort studies tracking biomarker trajectories and renal outcomes.

Compare cost-effectiveness and patient acceptability of combined screening strategies.

Integrate biomarker panels into risk stratification models alongside clinical variables.

Explore interventions targeting tubular injury when biomarkers indicate early damage.

### Conclusion

This study demonstrates that serum NGAL is a promising biomarker for early detection of diabetic nephropathy, offering better diagnostic accuracy than UACR alone. The combination of serum NGAL with UACR provides even stronger discrimination. These findings suggest that incorporating tubular injury markers could augment current screening, facilitating earlier intervention and potentially mitigating progression of renal disease in diabetic patients. However, given study limitations, further larger—especially longitudinal—studies are essential to validate predictive value and assess feasibility and cost-effectiveness in routine clinical practice. Nonetheless, our findings lay groundwork for enhancing DN screening strategies in settings like DDMCH, Keonjhar, and similar resource-limited environments.

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