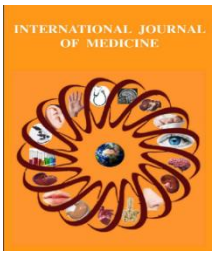

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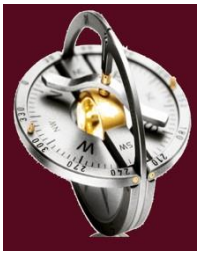

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Original Research

Urinary Tubular Injury Biomarkers and Systemic Inflammatory Markers in Chronic Kidney Disease: Correlation with Renal Function Decline and Clinical Outcomes at a Tertiary Care Teaching Centre.

Rayidi Manasa[1], Dr. Ritesh Vishwakarma[2*].
1Research Scholar Department of Biochemistry Index Medical College Hospital and Research Center Malwanchal University.
2Research Supervisor, Department of Biochemistry Index Medical College Hospital and Research Center Malwanchal University.

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Abstract

Background: Glomerular filtration rate (GFR) and albuminuria capture glomerular dysfunction but poorly reflect the tubulointerstitial injury and chronic low-grade inflammation that drive progressive nephron loss in chronic kidney disease (CKD). Urinary tubular injury markers and circulating inflammatory/oxidative-stress markers may provide complementary prognostic information. *Objective:* To characterize urinary neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), N-acetyl-β-D-glucosaminidase (NAG), liver-type fatty acid-binding protein (L-FABP), and β2-microglobulin, together with serum interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), hs-CRP, hepcidin, and asymmetric dimethylarginine (ADMA), across CKD stages 1-5 and controls, and to examine their relationship with renal function decline, anaemia, and 12-month clinical outcomes. *Methods:* In this prospective, observational, hospital-based cohort study, 280 participants (60 controls, 220 CKD patients stages 1-5) were recruited from a tertiary care teaching hospital in Indore, India, and followed for 12 months. Urinary and serum biomarkers were measured at baseline by ELISA, immunoturbidimetric, and spectrophotometric methods. *Results:* All urinary tubular markers and inflammatory markers rose progressively and significantly with advancing CKD stage ($p<0.001$ for all). Urinary NGAL and KIM-1 showed strong inverse correlations with eGFR ($r = -0.82$ and -0.78 respectively) and good discriminatory accuracy for 12-month progression (AUC 0.91 and 0.89). hs-CRP, IL-6, and TNF-α rose in parallel with declining haemoglobin, consistent with an inflammation-driven contribution to renal anaemia. Of 220 patients, 72 (32.7%) progressed at 12 months; progressors had markedly higher rates of dialysis initiation (25.0% vs 0%), major adverse cardiovascular events (13.9% vs 4.7%), hospitalization (33.3% vs 12.2%), and mortality (8.3% vs 1.4%; all $p<0.05$). *Conclusion:* Urinary tubular injury markers and systemic inflammatory markers increase in parallel with CKD severity and are strongly associated with disease progression and adverse clinical outcomes, supporting their use as complementary, non-glomerular indices of CKD risk alongside conventional filtration- and albuminuria-based assessment.

Keywords: *Chronic kidney disease; NGAL; KIM-1; tubular injury; inflammation; hs-CRP; progression.*

Introduction

Chronic kidney disease (CKD) is now recognized as a leading global cause of morbidity and mortality, with an estimated global prevalence of over 9% and more than a million deaths attributable to the condition in 2017 alone [1]. While the KDIGO 2012 classification system, based on cause, GFR category, and albuminuria category, has standardized CKD staging and remains the cornerstone of clinical assessment [2], it primarily reflects glomerular dysfunction and provides limited direct information about the tubulointerstitial compartment, which occupies over 90% of kidney parenchymal volume and is now understood to be a major, independent determinant of progression.

Tubulointerstitial injury and fibrosis correlate more closely with long-term renal outcomes in many nephropathies than glomerular changes alone, prompting substantial interest in urinary biomarkers that directly reflect tubular cell stress and damage. Kidney injury molecule-1 (KIM-1), a type I transmembrane glycoprotein markedly upregulated in dedifferentiated proximal tubular epithelial cells following injury, was first characterized as a sensitive and specific marker of proximal tubular damage and has since been established as the first kidney biomarker qualified by regulatory authorities for preclinical nephrotoxicity testing [3]. Neutrophil gelatinase-associated lipocalin (NGAL), synthesized and secreted by injured tubular epithelium, rises in both urine and plasma within hours of tubular insult, substantially earlier than serum creatinine, and has been extensively validated as an early, "troponin-like" biomarker of kidney injury in acute and chronic settings alike [4]. Complementary markers – NAG (reflecting proximal tubular lysosomal injury), L-FABP (reflecting oxidative stress in the proximal tubule), and β2-microglobulin (reflecting impaired tubular reabsorption of filtered low-molecular-weight proteins) – provide additional, partially non-overlapping windows into the tubular injury process.

Address for Correspondence Dr. Ritesh Vishwakarma, Research Supervisor, Department of Biochemistry Index Medical College Hospital and Research Center Malwanchal University.

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In parallel with tubular injury, chronic low-grade systemic inflammation is now recognized as both a consequence and a driver of CKD progression. Elevated pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), together with acute-phase reactants such as hs-CRP, have long been implicated in the pathogenesis of the malnutrition-inflammation-atherosclerosis (MIA) syndrome that characterizes advanced CKD and contributes to accelerated cardiovascular risk, protein-energy wasting, and mortality in this population [5]. Inflammatory cytokines also upregulate hepcidin, the master regulator of iron homeostasis, contributing to functional iron restriction and exacerbating renal anaemia independent of erythropoietin deficiency. Similarly, asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase that accumulates as renal clearance falls, links CKD-associated endothelial dysfunction to oxidative stress and cardiovascular risk.

Despite the growing mechanistic rationale for these markers, few studies from South Asian, and particularly Indian, tertiary care settings have simultaneously characterized the full panel of urinary tubular injury and systemic inflammatory markers across the complete spectrum of CKD stages with prospective clinical outcome data. This study was undertaken to address this gap: to quantify urinary NGAL, KIM-1, NAG, L-FABP, and β2-microglobulin, together with serum hs-CRP, IL-6, TNF-α, hepcidin, and ADMA, across CKD stages 1-5 and healthy controls; to correlate these markers with eGFR and haemoglobin; and to evaluate their association with 12-month CKD progression and clinical outcomes including major adverse cardiovascular events (MACE), hospitalization, and mortality.

Materials and Methods

Study design and setting: This prospective, observational, hospital-based cohort study was conducted jointly by the Departments of Biochemistry and Nephrology/General Medicine at Index Medical College, Hospital and Research Centre, Indore, Madhya Pradesh – a tertiary care teaching hospital equipped with dedicated nephrology and dialysis units and NABL-accredited biochemistry, immunoassay, and ELISA laboratories with cryopreservation (-80°C) facilities. The study spanned 24 months: 6 months for protocol development and ethics approval, 12 months for recruitment and sample collection, and 6 months for laboratory analysis and statistics.

Participants: Participants were enrolled from the Nephrology outpatient department, inpatient ward, and renal replacement therapy unit. CKD patients (18-70 years, KDIGO stages 1-5, stable renal function ≥3 months) and age-/sex-matched healthy controls (eGFR >90 mL/min/1.73m², UACR <10 mg/g, no diabetes or hypertension) were recruited after written informed consent. Individuals on maintenance dialysis, transplant recipients, those with acute kidney injury, active urinary tract infection, fever within two weeks, pregnancy, malignancy, or receiving corticosteroids/calcineurin inhibitors were excluded, since these conditions independently alter tubular injury and inflammatory marker levels.

Sample size: Based on an anticipated 12-month progression rate of ~30% in CKD stage 3-4 patients and an expected biomarker-progression correlation of r = 0.40, a minimum of 47 participants per group was calculated (α = 0.05, power = 80%); accounting for stage-wise sub-analysis and 20% attrition, the total target enrolment was 280 (30 in CKD1, 30 in CKD2, 60 each in CKD3 and CKD4, 40 in CKD5, and 60 controls).

Sample collection and assays: Fasting morning venous blood (~20 mL) and first-morning midstream urine (5 mL) were collected under aseptic conditions and processed within 2 hours. Serum was separated by centrifugation (3000 rpm, 10 minutes) and aliquots stored at -80°C for batch ELISA. Urinary NGAL, KIM-1, L-FABP, and β2-microglobulin, and serum IL-6, TNF-α, and hepcidin were measured by validated ELISA kits; urinary NAG by spectrophotometric assay; hs-CRP by latex-enhanced immunoturbidimetric assay; and ADMA by ELISA. eGFR was calculated by the CKD-EPI 2021 creatinine-cystatin C combined equation, and UACR by immunoturbidimetric assay.

Follow-up and outcomes: Patients were reassessed clinically and biochemically at 3, 6, and 12 months. The primary outcome – CKD progression – was defined as a sustained ≥40% decline in eGFR (confirmed on repeat testing ≥90 days apart) or initiation of renal replacement therapy. Secondary outcomes were MACE (non-fatal myocardial infarction, non-fatal stroke, cardiovascular death), worsening anaemia (haemoglobin <10 g/dL), and CKD-related hospitalization.

Statistical analysis: Analyses were performed using IBM SPSS v26.0 and MedCalc v20.1. Normality was assessed by the Shapiro-Wilk test; skewed biomarker data were log-transformed as required. Between-group comparisons used one-way ANOVA or Kruskal-Wallis tests with Bonferroni/Dunn post-hoc correction. Correlations were assessed by Pearson's or Spearman's coefficients. ROC curve analysis determined optimal cut-offs, sensitivity, specificity, and AUC. A p-value <0.05 was considered statistically significant.

Ethical considerations: Institutional Ethics Committee approval was obtained prior to initiation, and written informed consent (English/Hindi) was taken from all participants, with data anonymized and confidentiality maintained throughout.

Results

Table 1. Baseline Demographic Characteristics

Variable	Controls (n=60)	CKD 1 (n=30)	CKD 2 (n=30)	CKD 3 (n=60)	CKD 4 (n=60)	CKD 5 (n=40)	p-value
Age (years)	49.2±8.6	48.6±9.1	50.8±8.4	54.3±9.2	57.1±10.5	58.4±9.7	<0.001
Male (%)	58.3	60.0	63.3	65.0	68.3	70.0	0.41
Diabetes (%)	0	26.7	40.0	55.0	63.3	67.5	<0.001
Hypertension (%)	0	36.7	53.3	73.3	85.0	90.0	<0.001

The cohort showed the expected age-, diabetes-, and hypertension-related gradient across CKD stages, both established drivers of tubulointerstitial injury and systemic inflammation, providing appropriate context for interpreting the biomarker findings below.

Table 2. Selected Routine Parameters

Parameter	Controls	CKD1	CKD2	CKD3	CKD4	CKD5	p-value
eGFR (mL/min/1.73m²)	98±12	94±8	72±7	44±8	23±5	11±3	<0.001
UACR (mg/g)	7±3	42±18	115±54	364±141	821±268	1298±394	<0.001
Hemoglobin (g/dL)	13.8±1.1	13.2±1.2	12.4±1.3	11.2±1.4	9.8±1.5	8.4±1.6	<0.001

eGFR fell and UACR rose progressively across stages, confirming valid staging, while haemoglobin declined in step-wise fashion, providing the anaemia reference values against which the inflammatory marker findings (Table 3) are interpreted.

Table 3. Serum Inflammatory and Oxidative Stress Biomarkers

Biomarker	Controls	CKD1	CKD2	CKD3	CKD4	CKD5	p-value
hs-CRP (mg/L)	1.2±0.6	2.1±0.8	3.3±1.1	5.4±1.6	7.8±2.0	10.1±2.4	<0.001
IL-6 (pg/mL)	2.8±1.0	4.2±1.3	6.5±1.8	9.7±2.4	13.1±3.0	16.8±3.6	<0.001
TNF-α (pg/mL)	9.6±2.3	12.4±2.8	15.8±3.2	19.6±3.8	24.3±4.4	28.7±5.1	<0.001
Hepcidin (ng/mL)	18±6	24±7	31±8	45±10	58±12	71±15	<0.001
ADMA (µmol/L)	0.46±0.08	0.54±0.09	0.63±0.10	0.78±0.11	0.94±0.12	1.12±0.14	<0.001

hs-CRP, IL-6, and TNF-α rose progressively and in parallel with the fall in haemoglobin shown in Table 2, consistent with an inflammation-driven component of renal anaemia. Hepcidin elevation across stages further suggests progressive functional iron restriction secondary to

inflammatory cytokine-mediated upregulation, while the parallel rise in ADMA reflects worsening endothelial dysfunction as excretory clearance of this nitric oxide synthase inhibitor declines.

Table 4. Urinary Tubular Injury Biomarkers

Biomarker	Controls	CKD1	CKD2	CKD3	CKD4	CKD5	p-value
NGAL (ng/mL)	18±7	42±12	78±21	138±35	221±48	318±62	<0.001
KIM-1 (ng/mL)	0.74±0.22	1.31±0.34	2.14±0.51	3.48±0.79	5.26±1.08	7.34±1.36	<0.001
NAG (U/L)	4.6±1.1	6.9±1.5	9.8±2.1	14.6±3.0	20.3±4.2	27.8±5.1	<0.001
L-FABP (ng/mL)	5.1±1.4	8.8±2.0	13.2±2.8	20.8±4.1	31.6±5.6	44.2±7.8	<0.001
β2-Microglobulin (µg/L)	162±41	284±63	468±84	732±116	1054±168	1466±222	<0.001

All five urinary tubular markers rose progressively and significantly across CKD stages, with the steepest absolute increases seen from stage 3 onward. The magnitude of elevation was most pronounced for β2-microglobulin and NGAL, reflecting, respectively, impaired tubular reabsorptive capacity and active tubular cell injury/regeneration as nephron mass is progressively lost.

Table 5. Correlation of Urinary Tubular Markers with eGFR

Biomarker	r	p-value
NGAL	-0.82	<0.001
KIM-1	-0.78	<0.001

Both urinary NGAL and KIM-1 showed strong, statistically significant inverse correlations with eGFR, supporting their use as sensitive surrogates of tubular functional decline that parallel, but are mechanistically distinct from, glomerular filtration loss.

Table 7 (subset). ROC Analysis of Urinary Markers for Prediction of CKD Progression

Biomarker	Cut-off	Sensitivity (%)	Specificity (%)	AUC
NGAL	126 ng/mL	89.3	84.1	0.91
KIM-1	3.1 ng/mL	85.7	82.8	0.89

Urinary NGAL and KIM-1 both demonstrated good-to-excellent discriminatory accuracy for identifying patients likely to progress over 12 months, reinforcing their candidacy as adjuncts to conventional filtration-based risk assessment.

Table 9. Twelve-Month Clinical Outcomes

Outcome	Progressors (n=72)	Non-progressors (n=148)	p-value
Mean eGFR decline (%)	46.8±8.5	8.9±4.3	<0.001
Dialysis initiation	18 (25.0%)	0	<0.001
MACE	10 (13.9%)	7 (4.7%)	0.014
Hospitalization	24 (33.3%)	18 (12.2%)	<0.001
Mortality	6 (8.3%)	2 (1.4%)	0.018

Patients meeting the composite progression endpoint (32.7% of the CKD cohort) had markedly worse 12-month outcomes across every measured domain, underscoring that the elevated tubular injury and inflammatory marker profiles observed in this subgroup translate into clinically meaningful adverse events.

Discussion

This study demonstrates that urinary tubular injury markers and systemic inflammatory markers rise in a graded, statistically robust fashion across the CKD spectrum and correlate closely with both renal function decline and 12-month clinical outcomes in an Indian tertiary care cohort, providing evidence that these markers capture pathophysiological processes complementary to, and partly independent of, glomerular filtration loss.

The strong inverse correlation between urinary NGAL and eGFR, together with its good discriminatory performance for progression (AUC 0.91), is consistent with its established role as an early, sensitive marker of tubular epithelial stress; NGAL is upregulated and secreted by injured proximal and distal tubular cells well before measurable changes in filtration occur, a property first characterized in acute kidney injury but increasingly recognized as relevant to the sustained subclinical tubular injury that accompanies progressive CKD [4]. Similarly, KIM-1, first identified as a highly specific marker of dedifferentiated proximal tubular epithelium following injury, showed a comparable, though slightly less pronounced, relationship with both eGFR decline and progression risk (AUC 0.89), reinforcing its complementary role as a tubule-specific injury signal distinct from glomerular-based indices [3]. The concordant rise of NAG, L-FABP, and β2-microglobulin further supports a multi-mechanistic model of tubular injury in CKD, encompassing lysosomal enzyme leakage, oxidative stress, and impaired reabsorptive capacity respectively.

The parallel elevation of hs-CRP, IL-6, and TNF-α across CKD stages, together with their association with declining haemoglobin, is consistent with the well-described contribution of chronic low-grade inflammation to the pathogenesis of renal anaemia and the broader malnutrition-inflammation-atherosclerosis (MIA) syndrome that characterizes advanced CKD. Pro-inflammatory cytokines are known to blunt erythropoietin responsiveness and, via hepcidin upregulation, restrict iron availability for erythropoiesis — a mechanism reflected here by the progressive rise in serum hepcidin alongside falling haemoglobin [5]. This inflammatory milieu has additionally been linked to accelerated atherosclerosis and cardiovascular mortality in CKD, offering a plausible biological explanation for the significantly higher MACE rate observed among progressors in this cohort. The concurrent rise in ADMA is consistent with progressive endothelial dysfunction secondary to reduced renal clearance of this endogenous nitric oxide synthase inhibitor, providing a further mechanistic link between accumulating uraemic solutes, vascular injury, and the excess cardiovascular events observed in advanced CKD.

Taken together, these findings support a conceptual model in which glomerular injury (captured by eGFR/UACR), tubular injury (captured by NGAL, KIM-1, NAG, L-FABP, β2-microglobulin), and systemic inflammation (captured by hs-CRP, IL-6, TNF-α, hepcidin, ADMA) represent partially distinct but interacting pathophysiological axes, each contributing independently to disease trajectory and clinical outcome. This is consistent with the broader shift in CKD biomarker research toward multidimensional risk assessment rather than reliance on glomerular filtration surrogates alone.

Limitations: As a single-centre study with a 12-month follow-up window, findings may not generalize to other populations or longer time horizons; overlapping elevation of several markers in inflammatory and infective states not fully excluded by study criteria could confound interpretation in individual patients; and cost and assay standardization remain practical barriers to routine clinical adoption of this panel.

Conclusion

Urinary tubular injury biomarkers (NGAL, KIM-1, NAG, L-FABP, β2-microglobulin) and systemic inflammatory/oxidative-stress markers (hs-CRP, IL-6, TNF-α, hepcidin, ADMA) rise progressively with advancing CKD stage, correlate significantly with renal function decline and anaemia severity, and are strongly associated with 12-month disease progression and adverse clinical outcomes including dialysis initiation, MACE, hospitalization, and mortality. These markers provide mechanistically distinct, complementary prognostic information to conventional

glomerular-based assessment and merit further evaluation as components of integrated, multidimensional CKD risk-stratification tools in Indian clinical practice.

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