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Assessment Diagnostic Efficacy of NGAL, KIM-1, and MicroRNA Panels for Early Detection of Chronic Kidney Disease in Type 2 Diabetic Sudanese Patients

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Abstract

Background: *Chronic kidney disease (CKD) affects nearly 10% of the world's population and is a major cause of morbidity and mortality. Traditional diagnostic markers such as serum creatinine are insensitive to early renal injury. Emerging biomarkers—including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and microRNA panels (e.g., miR-451)—have shown promise for early detection. However, their diagnostic efficacy requires validation in resource-limited settings such as Sudan.*

Objectives: *This study aimed to evaluate the diagnostic performance of NGAL, KIM-1, and microRNA panels for detecting early renal injury in Type 2 diabetic Sudanese patients, and to compare these novel markers with conventional tests.*

Methods: *In this prospective cross-sectional study (2022–2024), 200 Type 2 diabetic patients without a previous CKD diagnosis were enrolled from several tertiary hospitals in Khartoum State. Patients were selected based on the presence of proteinuria or other early renal impairment indicators. NGAL was measured using a particle-enhanced turbidimetric immunoassay (PETIA), KIM-1 by sandwich ELISA, and microRNAs were quantified via RNA extraction, reverse transcription, and qPCR. Demographic, clinical, and laboratory data (including serum creatinine, eGFR, and cystatin C) were collected. Statistical analysis included descriptive statistics, t-tests/ANOVA, correlation analysis, logistic regression, and receiver operating characteristic (ROC) curve analysis.*

Results: *Patients with early kidney disease (n = 90) exhibited significantly higher levels of NGAL (160 ± 25 ng/mL vs. 130 ± 20 ng/mL, $p < 0.001$), KIM-1 (2300 ± 500 pg/mL vs. 1900 ± 400 pg/mL, $p < 0.005$), and microRNA levels (median 3.8 vs. 3.2 relative units, $p < 0.010$) compared to those without renal impairment. Individually, NGAL, KIM-1, and microRNA achieved AUCs of 0.87, 0.85, and 0.83, respectively. A combined biomarker model yielded an AUC of 0.90, with 88% sensitivity, 92% specificity, and a positive predictive value (PPV) of 89%. Multivariate logistic regression confirmed that increases in NGAL, KIM-1, and microRNA levels were significant predictors of early kidney disease.*

Conclusion: *A multimarker approach integrating NGAL, KIM-1, and microRNA panels markedly improves early CKD detection in Type 2 diabetic patients compared with conventional markers. The high diagnostic accuracy of the combined model supports its potential clinical application for timely intervention, especially in resource-limited settings.*

Keywords: Chronic kidney disease, NGAL, KIM-1, microRNA, early detection, diabetic nephropathy, Sudan.

INTRODUCTION

Chronic kidney disease (CKD) is characterized by a progressive decline in renal structure and function over a period of more than three months, often remaining asymptomatic during its early stages and leading to late diagnosis and missed opportunities for intervention (1,2). Approximately 10% of the global population is affected by CKD, and it is a leading contributor to morbidity and mortality worldwide. Traditional diagnostic markers such as serum creatinine and the estimated glomerular filtration rate (eGFR) are limited by their insensitivity in the early stages of kidney injury, necessitating the identification of novel biomarkers (3,4). Recent advances in molecular biology have identified several promising candidates for early detection. Neutrophil gelatinase-associated lipocalin (NGAL), a protein rapidly upregulated in response to tubular injury, has demonstrated high diagnostic accuracy in both plasma and urine, often preceding changes in serum creatinine (5,6). Similarly, Kidney Injury Molecule-1 (KIM-1) is a transmembrane protein expressed at low levels in healthy kidneys that is markedly upregulated following proximal tubular injury, offering an early indicator of renal damage (7,8). In addition, microRNAs (miRNAs)—small, non-coding RNA molecules involved in post-transcriptional regulation—have emerged as highly stable biomarkers with tissue specificity. For instance, miR-451 has shown potential for detecting early diabetic nephropathy, given its role in modulating inflammatory and fibrotic pathways (9,10). The National Heart, Lung, and Blood Institute first defined cardiorenal syndrome (CRS) in 2004 to emphasize the intrinsic interdependence of cardiac and renal pathologies (11,12). In regions like Sudan, where kidney disease is a leading cause of mortality and is frequently complicated by cardiovascular comorbidities (13,14), there is an urgent need for effective, noninvasive, and cost-effective diagnostic strategies. Moreover, in diabetic populations—a group at particularly high risk for CKD—the integration of novel biomarkers may facilitate earlier therapeutic interventions and improve patient outcomes (15,16). Against this backdrop, the current study aims to (i) evaluate the diagnostic performance of NGAL, KIM-1, and microRNA panels in detecting early renal injury among Type 2 diabetic patients in Khartoum State; (ii) determine diagnostic cut-off values specific to the Sudanese population; and (iii) assess the additive value of combining these biomarkers into a comprehensive risk stratification model. This multimarker approach is anticipated to yield a more precise and actionable assessment of renal injury, thereby enabling early intervention and potentially reducing the progression to end-stage renal disease (ESRD) (17,18).

MATERIALS AND METHODS

Study Design and Ethical Considerations

This study was conducted from 2022 to 2024 in multiple tertiary hospitals in Khartoum State, Sudan. Ethical approval was obtained from the Karary University Postgraduate Ethics Committee and the Sudan Ministry of Health Research Ethics Committee (Approval No: [Insert Approval No.]). Informed consent was obtained from all participants, and strict data protection protocols were observed throughout the study (10,11). Two hundred Type 2 diabetic patients without a prior diagnosis of CKD were enrolled. Inclusion criteria required evidence of early renal impairment, such as proteinuria, while patients with advanced CKD, known renal disease, or significant comorbidities were excluded. The sample size was calculated based on a population estimate of 2,454,701 (from Khartoum's census), a 5% margin of error, and a 95% confidence interval. Demographic and clinical data, including age, gender, residence, duration of diabetes, and treatment modalities, were collected. Laboratory tests included serum creatinine, cystatin C, and urine protein analysis. eGFR was calculated using the CKD-EPI equation. Additionally, NGAL, KIM-1, and microRNA levels were measured as described below. Blood and urine samples were collected using standard protocols. NGAL was quantified using a particle-enhanced turbidimetric immunoassay (PETIA) on the Roche cobas e601 analyzer. The assay, with an analytical range of 50–3000 ng/mL, provided results within approximately 10 minutes. Samples were maintained at 2–8°C during transport and processed within 6 days if refrigerated. KIM-1 levels were measured in urine using a sandwich enzyme-linked immunosorbent assay (ELISA) with horseradish peroxidase detection. The assay's analytical range was 0.5–50 ng/mL (converted from pg/mL), with colorimetric detection at 450 nm. Each assay run included calibration using standard kits, and samples were processed within 2–3 hours. Total RNA, including microRNAs, was extracted from plasma and urine samples using TRIzol reagent and processed with a Qiagen QIAcube Connect system. Reverse transcription was conducted to synthesize cDNA, followed by quantitative PCR (qPCR) with normalization to housekeeping miRNAs. The entire process required approximately 3 hours, and results were expressed in relative units. Renal function was evaluated using the estimated glomerular filtration rate (eGFR), calculated from serum creatinine and cystatin C levels using validated equations (32–35). Serum creatinine was measured on a Beckman Coulter AU5800 Chemistry Analyzer. Data were analyzed using SPSS version 26 (IBM Inc., Chicago, IL, USA). Continuous variables were expressed as means ± standard deviations (SD) or medians (interquartile ranges, IQR) and compared using independent t-tests or Mann-Whitney U tests. Categorical variables were summarized as frequencies and percentages and analyzed using Chi-square or Fisher's exact tests. Pearson or Spearman correlation coefficients were used for bivariate analyses. Multivariate logistic regression identified independent predictors of early kidney disease, and ROC curve analyses were performed to assess the diagnostic performance of individual and combined biomarkers. A two-tailed p-value < 0.05 was considered statistically significant. All laboratory personnel adhered to Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) standards. Equipment calibration, standardized protocols for sample collection, storage at –80°C, and strict biosafety measures (including use of gloves, lab coats, and face shields) were maintained.

RESULTS

The study enrolled 200 Type 2 diabetic patients with a mean age of 58 ± 12 years; the gender distribution was even (50% male, 50% female). Approximately 65% resided in urban areas, and 60% were insulin dependent. The mean duration of diabetes was 15 ± 5 years. Twenty-five percent of patients had a history of renal disease. Baseline laboratory assessments revealed that the mean NGAL level was 145 ± 30 ng/mL, KIM-1 was 2100 ± 600 pg/mL, and microRNA expression was 3.5 ± 1.0 relative units. Patients with early kidney disease (n = 90) had significantly higher NGAL (160 ± 25 ng/mL vs. 130 ± 20 ng/mL, p < 0.001), KIM-1 (2300 ± 500 pg/mL vs. 1900 ± 400 pg/mL, p < 0.005), and microRNA levels (median 3.8 [IQR: 3.4–4.2] vs. 3.2 [IQR: 2.8–3.6], p < 0.010) compared to those without renal impairment (**Table 2**, integrated in text). ROC curve analyses demonstrated that individually, NGAL, KIM-1, and microRNA achieved AUCs of 0.87, 0.85, and 0.83, respectively. Notably, a combined model incorporating all three biomarkers significantly improved diagnostic performance, achieving an AUC of 0.90, with 88% sensitivity, 92% specificity, and a positive predictive value (PPV) of 89% (**Table 4**). Multivariate logistic regression confirmed that increases in NGAL (OR 1.25 per 10 ng/mL), KIM-1 (OR 1.10 per 100 pg/mL), and microRNA (OR 1.50 per unit) were significant independent predictors of early kidney disease (p < 0.001) (**Table 12**).

Integration of Key Tables in Results

Below are the four selected tables drawn from the original document, presented exactly as they appeared, with explanations linking them to the "Results" section:

Table 1. Demographic and Clinical Characteristics of the Study Population (n = 200)

Variable	Category	Value / Count (%)
Age (years)	Mean ± SD (Range)	58 ± 12 (31 – 79)
Gender	Male	100 (50%)
	Female	100 (50%)
Residence	Khartoum	100 (50%)
	Port Sudan	60 (30%)
	Bahri	40 (20%)
Occupation	Employed	90 (45%)
	Unemployed	60 (30%)
	Retired	40 (20%)
	Student	10 (5%)
Diabetes Treatment	Insulin	120 (60%)
	Oral	50 (25%)
	Diet	30 (15%)
Duration of Diabetes (yrs)	Mean ± SD (Range)	15 ± 5 (5 – 30)
History of Renal Disease	Yes	50 (25%)
	No	150 (75%)

This table supports the demographic and clinical description in the "Results" section, confirming the sample's representativeness (mean age 58 ± 12 years, 50% male/female, 60% insulin-dependent, 25% with renal disease history). It provides context for interpreting biomarker differences between those with and without early kidney disease.

Table 2. Comparison of Biomarker Levels Between Patients With and Without Early Kidney Disease

Biomarker	Group	Mean ± SD or Median (IQR)	p-value
NGAL (ng/mL)	Early Kidney Disease (n=90)	160 ± 25	<0.001
	No Kidney Disease (n=110)	130 ± 20	
KIM-1 (pg/mL)	Early Kidney Disease (n=90)	2300 ± 500	<0.005
	No Kidney Disease (n=110)	1900 ± 400	
MicroRNA (Relative Units)	Early Kidney Disease (n=90)	3.8 (3.4–4.2)	<0.010
	No Kidney Disease (n=110)	3.2 (2.8–3.6)	

This table directly corresponds to the statement in the "Results" section: "Patients with early kidney disease (n = 90) had significantly higher NGAL (160 ± 25 ng/mL vs. 130 ± 20 ng/mL, p < 0.001), KIM-1 (2300 ± 500 pg/mL vs. 1900 ± 400 pg/mL, p < 0.005), and microRNA levels (median 3.8 [IQR: 3.4–4.2] vs. 3.2 [IQR: 2.8–3.6], p < 0.010)." It quantifies the biomarker differences, reinforcing their diagnostic potential.

Table 3. Multivariate Logistic Regression Analysis Predicting Early Kidney Disease

Variable	Odds Ratio (OR)	95% CI	p-value
NGAL (per 10 ng/mL)	1.25	1.15–1.36	<0.001
KIM-1 (per 100 pg/mL)	1.10	1.05–1.15	<0.001
MicroRNA (per unit)	1.50	1.20–1.88	<0.001
Age (per year)	1.02	0.99–1.05	0.18
Systolic BP (per mmHg)	1.03	1.01–1.05	0.01
Diabetes Duration (per year)	1.04	0.98–1.10	0.20

Link to Results: This table aligns with the "Results" section's statement: "Multivariate logistic regression confirmed that increases in NGAL (OR 1.25 per 10 ng/mL), KIM-1 (OR 1.10 per 100 pg/mL), and microRNA (OR 1.50 per unit) were significant independent predictors of early kidney disease (p < 0.001)." It provides the statistical evidence for their predictive power.

Table 4. ROC Analysis for the Combined Biomarker Model

Metric	Value (%)
AUC	0.90
Sensitivity	88
Specificity	92
PPV	89
NPV	91

Link to Results: This table directly supports the "Results" section's claim: "A combined model incorporating all three biomarkers significantly improved diagnostic performance, achieving an AUC of 0.90, with 88% sensitivity, 92% specificity, and a positive predictive value (PPV) of 89%." It quantifies the superior diagnostic accuracy of the multimarker approach.

DISCUSSION

Chronic kidney disease (CKD) is a pressing global health problem, particularly in low-resource settings such as Sudan, where limited access to advanced diagnostic and treatment modalities leads to late diagnosis and rapid progression of kidney dysfunction (1–4). Early detection is critical, as traditional markers like serum creatinine are insufficiently sensitive to subclinical renal injury. In this context, novel biomarkers—NGAL, KIM-1, and microRNA panels—offer the potential for earlier, more precise detection. Our study confirmed that NGAL and KIM-1, which reflect tubular injury, are significantly elevated in Type 2 diabetic patients with early kidney disease compared with those without renal impairment (**Table 2**). NGAL, measured via PETIA, exhibited an AUC of 0.87 with high sensitivity (84%) and specificity (88%). This is in agreement with prior studies demonstrating that NGAL rises rapidly following tubular damage, often preceding creatinine changes (5,9,29). Similarly, KIM-1, a marker specific to proximal tubule injury, demonstrated an AUC of 0.85 with comparable sensitivity and specificity, corroborating its diagnostic value as reported by Han et al. (42) and Chua et al. (53). Moreover, microRNA profiling particularly panels including miR-451 further enhanced diagnostic accuracy. Individual microRNAs showed promising diagnostic performance (AUC of 0.83); however, when combined into a panel, the diagnostic yield improved (AUC ~0.91 in literature) (7,35,71). Our combined multimarker model, integrating NGAL, KIM-1, and microRNA data, achieved an AUC of 0.90, with sensitivity of 88% and specificity of 92%, underscoring the benefit of a composite approach in capturing the multifactorial pathophysiology of CKD (22,58) (**Table 4**). Multivariate logistic regression analysis identified NGAL, KIM-1, and microRNA levels as independent predictors of early kidney disease, even after adjusting for confounders such as age, systolic blood pressure, and diabetes duration (**Table 3**). Notably, the odds ratios suggest that even modest increases in these biomarkers are associated with a significant elevation in risk, highlighting their potential to serve as early warning indicators (17,18). Our findings are particularly relevant for the Sudanese context, where CKD is the sixth leading cause of death (13,15). The region-specific data reveal that genetic, environmental, and lifestyle factors may influence biomarker thresholds; hence, locally validated cut-offs are essential for accurate diagnosis and risk stratification (10–13). The use of advanced data visualization—such as pie charts for demographic distributions (Figure 1) and the ROC curve for the combined model (Figure 5)—provided clinicians with intuitive tools to interpret complex biomarker data in real time. Despite the clear advantages, challenges remain. The technical complexity and cost of microRNA assays necessitate further standardization and automation before routine clinical use. Similarly, although NGAL and KIM-1 assays have demonstrated high analytical performance, integrating them into existing diagnostic workflows in resource-limited settings will require additional operational and economic evaluations (40,47,76). In conclusion, our study provides compelling evidence that a multimarker approach incorporating NGAL, KIM-1, and microRNA panels significantly enhances early detection of CKD in high-risk Type 2 diabetic patients. This strategy holds promise for initiating early interventions, tailoring treatments, and ultimately improving patient outcomes in resource-limited settings.

CONCLUSIONS

This study demonstrates that NGAL, KIM-1, and microRNA panels are highly effective biomarkers for the early detection of CKD in Type 2 diabetic patients. Our multimarker model achieved a diagnostic AUC of 0.90, significantly outperforming individual markers. These findings support the incorporation of these novel biomarkers into routine clinical practice, especially in regions with limited resources, to enable earlier interventions and improve long-term outcomes.

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