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ORIGINAL ARTICLE

Optimized multiparametric approach for early detection of kidney disease in diabetic patients

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Abstract

Background Diabetic Kidney Disease (DKD) is a serious complication of diabetes. Identifying high-risk DKD patients can lead to better clinical outcomes.

Objective This study aimed to investigate the relevance of three potential predictive markers for DKD: Kidney Injury Molecule-1 (KIM-1), Tumor Necrosis Factor Receptor 1 (TNFR1), and urinary albumin-to-creatinine ratio (ACR).

Methods We recruited 120 participants, including 30 individuals with type 2 diabetes (T2D) but no renal complications, 30 with DKD, and 60 healthy controls. Blood and urine analyses were performed to assess lipid and liver parameters, ACR, and estimated glomerular filtration rate (eGFR). Plasma levels of KIM-1 and TNFR1 were measured using the sandwich ELISA method.

Results The results demonstrated that KIM-1 ($p = 0.008$) and TNFR1 ($p = 0.006$) levels were significantly higher in individuals with T2D compared to the controls and even more elevated in those with DKD ($p = 0.003$ and $p = 0.000$, respectively). KIM-1 ($p = 0.000$) and TNFR1 ($p = 0.001$) levels were significantly elevated in individuals with T2D without elevated albuminuria compared to control. KIM-1 and TNFR1 exhibited correlations with ACR ($r = 0.400$; $p = 0.002$ and $r = 0.607$; $p = 0.000$, respectively) and eGFR ($r = -0.425$; $p = 0.001$ and $r = -0.661$; $p = 0.000$, respectively). The ROC curve analysis revealed an area under the curve (AUC) of 0.91 for TNFR1, 0.76 for KIM-1, and 0.95 for ACR. However, the ACR, KIM-1, and TNFR1 combination showed the best predictive performance with an AUC of 0.98.

Conclusion Plasma levels of KIM-1 and TNFR1 are promising biomarkers for predicting kidney disease in individuals with diabetes, and their combination with ACR improves the overall diagnostic accuracy.

Keywords Diabetic kidney disease · Early diagnosis · ACR · KIM-1 · TNFR1 · Benin

Abbreviations

ACR	Albumine/Creatinine Ratio
AUC	Area Under Curve
BMI	Body Mass Index
DKD	Diabetic Kidney Disease
eGFR	Glomerular Filtration Rate estimated
KDOQI	Kidney Disease Outcomes Quality Initiative
KIM-1	Kidney Injury Molecule-1
OR	Odds Ratio
ROC	Receiver Operating Characteristic
T2D	Type 2 Diabetes
TNFR1	Tumor Necrosis Factor Receptor-1

Introduction

Diabetic kidney disease (DKD) is a serious complication of diabetes that affects a large number of both type 1 (T1D) and type 2 diabetes (T2D) patients [1, 2], potentially leading to end-stage renal disease (ESRD) and increasing cardiovascular risks [3].

In Africa, the prevalence of DKD varies widely from 11% to 83.7% [4], with high incidence rates reported in Benin [5]. DKD is characterized by a glomerular filtration rate (GFR)

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of less than 60 mL/min/1.73 m^2 and/or the presence of albuminuria [6]. However, albuminuria, which is the most commonly used early clinical indicator of renal insufficiency, has some limitations as a biomarker [7]. It may only reveal renal dysfunction after a long period of silent disease [8]. More than 50% of diabetic patients with chronic renal insufficiency have no previous history of albuminuria, as shown by epidemiological surveys and clinical trials like NHANES [9]. Additionally, patients with microalbuminuria may progress to macroalbuminuria or return to normoalbuminuria. [10]. Therefore, identifying other biomarkers of renal function is crucial to accurately predict the development and progression of DKD [7, 11].

New research suggested that multiple pathways, such as inflammation and tubular lesions, contribute to the progression of diabetic kidney disease (DKD) [11, 12]. One key factor in the development of DKD is the pro-inflammatory cytokine tumor necrosis factor (TNF)-α [12]. In both Caucasians and Pima Indians with diabetes, the reliable predictors of renal insufficiency are the circulating soluble TNF receptors, TNFR1 and TNFR2 [13, 14]. These TNF receptors have also been shown to predict end-stage renal disease (ESRD) in patients with T2D over a decade, as well as the onset of kidney disease in those with type 1 diabetes (T1D) [13, 14]. Another promising biomolecule, kidney injury molecule-1 (KIM-1), is a type I transmembrane glycoprotein expressed in response to renal proximal tubular cell injury, promoting kidney fibrosis [15]. KIM-1 can be detected in urine and the bloodstream following proximal tubule damage and has shown sensitivity and specificity as a marker for renal lesions, making it a valuable predictor of prognosis [16]. In individuals with T1D, plasma KIM-1 levels predicted a decline in glomerular filtration rate (GFR) and the risk of ESRD over a ten-year follow-up period [15]. Our study aims to evaluate the predictive value of KIM-1 and TNFR1 as markers of DKD in T2D patients and assess the diagnostic performance of combined KIM-1, TNFR1, and albuminuria markers. By comparing KIM-1 and TNFR1 biomarkers with albuminuria, which is commonly used to diagnose DKD, we aim to optimize the detection of DKD and provide new avenues for early diagnosis of renal disease in diabetics.

Materials and methods

Study design

This is an observational, analytical, case–control study conducted jointly at the Insulin Bank and the Nephrology Department of Hubert Koutoukou Maga University Hospital in Cotonou. The study population consisted of two independent groups of T2D patients (age > 35 years) with (n = 30) and without (n = 30) kidney disease. Additionally, a control group of 60 non-diabetic subjects with no renal disease and no history of renal failure was recruited during a screening campaign for diabetes, hypertension, and renal failure. The sample size of 30 participants per group was chosen to ensure sufficient power to detect meaningful differences in biomarker levels, based on established practices in similar biomedical research studies [17]. Participants were recruited using a convenience sampling method, selecting subjects based on their availability and willingness to participate while ensuring they met the inclusion criteria.

Inclusion criteria

Type 2 diabetes (T2D) patients

Individuals diagnosed with type 2 diabetes based on fasting blood glucose levels greater than 1.26 g/L (7 mmol/L) after an 8–12 h fast, confirmed on two separate occasions, or the presence of diabetes symptoms (polyuria, polydipsia, weight loss) associated with a blood glucose level higher than 2 g/L, in accordance with WHO criteria [18].

Diabetic kidney disease (DKD) patients

Individuals with T2D and a glomerular filtration rate (GFR) of < 60 ml/min/1.73 m^2 and/or a continuous increase in albumin-to-creatinine ratio (ACR) (> 30 mg/g) over three months.

Control group

Non-diabetic individuals without kidney disease and no history of renal insufficiency.

Exclusion criteria

Pregnant women, individuals with urinary tract infections, fever, infectious diseases, cancer, those following a protein-rich diet or having undergone surgery within the last 30 days were excluded from the study.

Definition of diabetes, diabetic kidney disease, and hypertension

Diabetes was defined as a fasting blood glucose level greater than 1.26 g/L (7 mmol/L) after an eight to twelve-hour fast and confirmed on two separate occasions, or the presence of diabetes symptoms (polyuria, polydipsia, weight loss) associated with a blood glucose level higher than 2 g/L in accordance with the WHO criteria [18]. Diabetic kidney

disease was defined as a glomerular filtration rate (GFR) of < 60 ml/min/1.73 m^2 and/or a continuous increase in albumin-to-creatinine ratio (ACR) (> 30 mg/g) over three months in a patient with T2D and no other types of kidney disease [19]. Hypertension was defined as systolic blood pressure of > 140 mm Hg and diastolic pressure of > 90 mm Hg or the use of antihypertensive medication.

Anthropometric data

Age, gender, medical history (including hypertension, cardiovascular conditions, smoking, medication), and family history were gathered through a questionnaire utilizing KoboToolbox and by reviewing medical records. Height, weight, and blood pressure were measured using standardized methods. Blood pressure was assessed twice, and the average recorded. Body mass index (BMI) was computed as weight in kilograms divided by height in meters squared.

Biochemical data

Participants provided venous blood samples after fasting overnight, which were collected in both dry tubes and tubes containing anticoagulant and antiglycolytic agents. The plasma and serum were separated, divided into aliquots, and stored at -20 °C until analyzed. Urine samples were used to determine the albumin/creatinine ratio. Using an automated biochemical analyzer with kits from ELITech (ELITech Group, France), the following were assessed: fasting blood glucose (FG), glycosylated hemoglobin (HbA1c), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and albumin. Kits from Bio-Techne (Abingdon, Oxon, UK) were used to quantify fasting serum KIM-1 (Human Serum TIM-1/KIM-1/HAVCR Quantikine ELISA Kit; #DSKM100) and TNFR1 (Human TNF RI/TNFRSF1A Quantikine QuicKit ELISA; #QK225) levels. Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald equation [20]. The estimated glomerular filtration rate (eGFR) was determined using the Modification of Diet in Renal Disease (MDRD) formula.

Statistical analysis

Statistical analysis was performed using SigmaPlot 14.0 software. Mean values with standard errors were utilized for quantitative variables, while percentages were used for qualitative variables. The choice between ANOVA or Kruskal–Wallis tests depended on the distribution of the data. Categorical variables are expressed as percentages. The Chi-squared test was used to compare categorical variables between groups. To explore relationships between variables, the Spearman correlation coefficient was utilized. Odds ratios and 95% confidence intervals were calculated by logistic regression to assess associations between biomarkers and diabetic kidney disease (DKD). ROC curves were generated to assess the predictive capabilities of biomarkers and their combined impact on DKD. Statistical significance was established at $p < 0.05$.

Results

Participants characteristics

Baseline characteristics of the study participants are summarized in Table 1. There were no statistically significant differences between groups for parameters such as sex, BMI, waist circumference, blood pressure, diabetes treatment, family history of kidney disease, smoking, alcohol consumption and physical activity (Table 1). However, individuals with T2D ($p = 0.000$) and those with DKD ($p = 0.000$) were notably older than the controls (Table 1).

The prevalence of a family history of diabetes was significantly higher in T2D patients ($p = 0.000$) and in those with DKD ($p = 0.000$) compared to controls. Additionally, subjects with DKD had a significantly longer duration of T2D ($p = 0.016$) than those with T2D alone. Hypertension was more common in the DKD group compared to both the control ($p = 0.000$) and T2D ($p = 0.014$) groups (Table 1).

Fasting blood glucose was significantly increased in both T2D ($p = 0.000$) and DKD subjects ($p = 0.000$) compared to controls (Table 2). TC levels were significantly higher in T2D ($p = 0.028$) compared to controls, as were HDL-c levels ($p = 0.000$). However, HDL-c levels were decreased in DKD ($p = 0.000$) compared to T2D subjects. LDL-c and TG levels showed no significant differences between the groups (Table 2).

Liver markers (ASAT and ALAT) and total proteins levels were comparable between the studied groups (Table 2). There were statistically significant differences in serum creatinine levels ($p = 0.000$), urinary albumin ($p = 0.000$), along with a decline in eGFR levels ($p = 0.000$) among DKD patients, compared to both T2D and control group (Table 2).

KIM-1 levels were significantly elevated in both T2D ($p = 0.008$) and DKD subjects ($p = 0.000$) compared to controls (Table 2). Similarly, TNFR1 levels were significantly higher in T2D ($p = 0.006$) and DKD subjects ($p = 0.000$) (Table 2). Notably, both KIM-1 levels ($p = 0.003$) and TNFR1 levels ($p = 0.000$) were significantly higher in DKD subjects compared to those with T2D alone (Table 2).

Table 1 Socio-anthropometric characteristics of the study population

Characteristics	Control (N = 60)	T2D (N = 30)	DKD (N = 30)	*p*
Age (years)	46.96 ± 2.18	56.70 ± 1.38[a***]	61.97 ± 1.73[a***]	0.000(1)
Gender, n(%)				
Female	29(48.0)	20(66.7)	14(46.7)	0.229(2)
Male	31(52.0)	10(33.3)	16(53.3)	
BMI, kg/m^2	25.64 ± 1.12	28.40 ± 0.78	27.05 ± 0.96	0.134(1)
Waist size, cm	92.36 ± 3.01	98.9 ± 2.30	99.78 ± 2.19	0.087(1)
Systolic BP, mmHg	130.24 ± 4.78	121.13 ± 2.24	127.90 ± 3.79	0.398(3)
Diastolic BP, mmHg	85.36 ± 3.27	79.03 ± 1.60	77.23 ± 1.82	0.135(3)
Pulses, bpm	74.40 ± 2.23	81.47 ± 1.83	82 0.03 ± 3.20	0.053(1)
T2D duration, years	-	10.58 ± 1.48	16.06 ± 1.80[b*]	0.016(2)
Insulin, n(%)	-	9(30.0)	5(16.7)	0.398(2)
OAD, n(%)	-	26(86.7)	20(71.4)	0.268(2)
Family history of T2D, n(%)	5(8.0)	22(73.3)[a***]	21(70.0)[a***]	0.000(2)
Family history of KD, n(%)	0(0.0)	2(7.1)	4(14.3)	0.082(2)
Hypertension, n(%)	12(20.0)	14(46.4)	22(75.0)[a***b*]	0.000(2)
Hypertension treatment, n(%)	5(8.0)	13(42.9)	24(75.0)	0.001(2)
Cardiovascular complications, n(%)	0(0.0)	0(0.0)	5(17.2)	-
Stroke	0(0.0)	1(3.3)	0(0.0)	
Myocardial Infection				
Smoking history, n(%)	2(4.0)	1(3.6)	1(3.6)	0.313(2)
Alcohol, n(%)	0(0.0)	3(10.7)	3(10.7)	0.248(2)
Past consumers	5(8.0)	0(0.0)	1(3.6)	
Frequent	29(48.0)	19(60.7)	18(64.3)	
Never	26(44.0)	8(28.6)	8(21.4)	
Occasional				
Physical activity, n(%)	29(48.0)	16(57.1)	12(35.7)	0.273(2)

Results are presented as mean ± SEM for continuous variables and as n(%) for categorical variables; T2D, type 2 diabetic subjects without kidney disease; DKD, diabetic subjects with kidney disease; BMI, body mass index; BP, blood pressure; OAD, oral antidiabetics drugs; KD, kidney disease. Comparisons of means and proportions between different groups were performed by (1) ANOVA; (2) Chi-2; (3) Kruskal–wallis tests. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (as showed in *p value* column). [a]significance vs. control; [b]Significance vs. T2D

Association between KIM-1 and TNFR1 and conventional markers of DKD

We stratified KIM-1 and TNFR1 markers based on ACR and eGFR levels in study participants. KIM-1 and TNFR1 levels were significantly increased in patients compared to control group (Fig. 1a). Furthermore, TNFR1 levels significantly increased with rising ACR levels, unlike KIM-1 (Fig. 1a). As the eGFR rate decreased, there was significant elevation in TNFR1 levels (Fig. 1b). Compared to eGFR levels, the variation in KIM-1 was stage-specific, with a significant increase shown at $30 \leq$ eGFR < 60 ml/min/1.73m^2 compared to other eGFR levels (Fig. 1b).

ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; TNFR1: tumor necrosis factor receptor 1. KIM-1 and TNFR1 levels were distributed according to ACR and eGFR levels. Results are expressed as mean ± SEM. Comparison between means was performed by post-hoc analysis (Dunn's method). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Correlation between KIM-1 and TNFR1 with T2D and kidney disease diagnosis parameters

In our study, KIM-1 demonstrated a significant positive correlation with both the duration of diabetes (r = 0.302; $p = 0.019$) and the albumin/creatinine ratio (ACR) (r = 0.400; $p = 0.002$) (Table 3). Conversely, KIM-1 exhibited a negative correlation with the estimated glomerular filtration rate (eGFR) (r = -0.425, $p = 0.001$) (Table 3). Similarly, TNFR1 levels were significantly positively correlated with age (r = 0.267; $p = 0.039$), duration of diabetes (r = 0.316; $p = 0.014$), and ACR (r = 0.607; $p = 0.000$), while showing a strong negative correlation with eGFR (r = -0.661; $p = 0.000$) (Table 3). The analysis also revealed a significant positive correlation between KIM-1 and TNFR1 (r = 0.590; $p = 0.000$) (Table 3).

Table 2 Clinical characteristics of the study population

Parameters	Control(n = 60)	T2D(n = 30)	DKD(n = 30)	*p*
FG(g/L)	0.81 ± 0.02	1.44 ± 0.11^{a***}	1.54 ± 0.17^{a***}	0.000
HbA1C(%)	-	8.56 ± 0.79	8.17 ± 0.58	0.824
TC(g/L)	1.71 ± 0.09	2.08 ± 0.07^{a*}	1.98 ± 0.11	0.033
HDL-c(g/L)	0.33 ± 0.04	0.57 ± 0.04^{a***}	0.47 ± 0.03^{b***}	0.000
LDL-c(g/L)	1.19 ± 0.10	1.33 ± 0.07	1.29 ± 0.12	0.516
TG(g/L)	0.98 ± 0.06	0.96 ± 0.05	1.069 ± 0.05	0.129
Creatinine(mg/L)	8.66 ± 0.28	8.64 ± 0.26	14.06 ± 1.25^{ab***}	0.000
TP(g/L)	75.77 ± 1.05	76.73 ± 1.22	78.20 ± 1.43	0.317
ALT(IU/L)	21.04 ± 2.02	17.36 ± 3.70	12.49 ± 1.93	0.029
AST(IU/L)	28.57 ± 2.16	29.83 ± 5.19	19.83 ± 3.10	0.031
ACR(mg/g)	23.68 ± 4.12	13.80 ± 1.35	282.57 ± 51.59^{a*b***}	0.000
eGFR(ml/min/1.73m^2)	101.97 ± 2.30	94.16 ± 3.39	66.92 ± 5.16^{a***b**}	0.000
KIM-1(pg/ml)	33.16 ± 3.58	64.73 ± 7.54^{a*}	221.10 ± 43.63^{a***b**}	0.000
TNFR1(pg/ml)	1254.56 ± 52.79	1613.77 ± 76.61^{a**}	3166.53 ± 232.66^{ab***}	0.000

Results are presented as mean ± SEM; T2D, subjects with type 2 diabetes; DKD, diabetic subjects with kidney disease; FG, fasting glucose; TC, total cholesterol; HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; TG, triglycerides; PT, total protein ALT, alanine amino transferase; AST, aspartate amino transferase; ACR, albumin/creatinine ratio; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate according to the MDRD formula; KIM-1, Kidney injury molecule-1; TNFR1, Tumor necrosis factor receptor 1. Comparison between means was performed by post-hoc analysis (Dunn's method). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; [a]significance vs. control; [b]significance vs. T2D

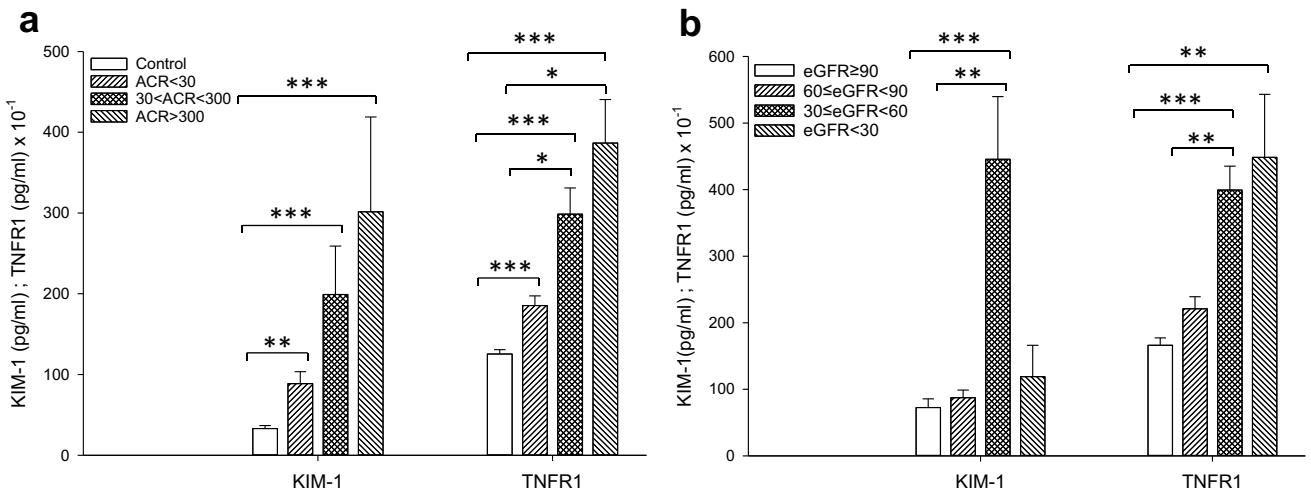


Fig. 1 KIM-1 and TNFR1 compared to **a** albumin/creatinine ratio and **b** estimated glomerular filtration rate levels

Multivariable logistic regression

Multivariable logistic regression adjusted for age, sex, and BMI, duration of diabetes, hypertension, and HDL-c, showed that KIM-1, TNFR1, and ACR were independently associated with DKD (OR: 1.021; 95% CI: 1.006–1.037; $p = 0.006$), (OR: 1.004; 95% CI: 1.001–1.006; $p = 0.003$), and (OR: 1.182; 95% CI: 1.035–1.349; $p = 0.013$) respectively (Table 4).

Diagnosis performance

The ROC curve showed that the area under the curve (AUC) value for KIM-1 was 0.76 (95% CI; 0.64–0.89; $p = 0.000$), discriminating the renal outcome with 73.33% sensitivity and 76.67% specificity at a cut-off value of 71.13 pg/ml (Fig. 2). The AUC value for TNFR1 was 0.91 (95% CI; 0.83–0.98; $p = 0.000$), with 90.00% sensitivity and 86.67% specificity at a 1868 pg/ml cut-off (Fig. 2). ACR had an AUC of 0.95 (95% CI; 0.90–1.00; $p = 0.000$) (Fig. 2). A level of

Table 3 Spearman correlation between KIM-1 and TNFR1 with T2D and kidney disease diagnosis parameters

Parameters	KIM-1	TNFR1	ACR	eGFR
Age	0.244	0.267*	0.287*	-0.441***
BMI	0.044	-0.031	-0.046	-0.098
T2D duration	0.302*	0.316*	0.161	-0.379**
HbA1c	-0.300	-0.321	-0.106	0.298
eGFR	-0.425***	-0.661***	-0.418**	-
ACR	0.400**	0.607***	-	-
TNFR1	0.590***	-	-	-

KIM-1, kidney injury molecule-1; TNFR1, tumor necrosis factor receptor 1; ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate; BMI, body mass index; T2D, type 2 diabetes; HbA1c, glycated hemoglobin. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

ACR > 29.63 mg/g distinguishes renal disease with 73.33% sensitivity and 96.67% specificity. Moreover, our results demonstrated that an ACR value of 17.42 mg/g may be an optimal cut-off, discriminating renal disease with 90.00% sensitivity and 80.00% specificity. The combination of the three markers significantly improved discrimination for the kidney outcome than any of them considered individually, with an AUC of 0.98 (95% CI; 0.96–1.00; $p = 0.000$), 90.00% sensitivity, and 96.67% specificity (Fig. 2).

Discussion

Diabetic Kidney Disease (DKD) is a serious complication of diabetes that can lead to high rates of illness and death [1]. Detecting it early and providing protective treatments are crucial for slowing down the progression of DKD and improving the outlook for patients. However, the traditional clinical marker for DKD, albuminuria, has limitations [7], and there is a pressing need for new predictive biomarkers. Recent studies have shown that inflammation and tubular lesions play a significant role in DKD progression [7, 21]. KIM-1, a protein expressed in renal tubule cells, helps with tissue repair and regeneration [22], while TNFR1, a cytokine receptor, is involved in inflammation and cell death [23]. Both markers were significantly associated with DKD progression [7]. In our study, we tested the relevance of circulating KIM-1 and TNFR1 levels as predictive biomarkers for DKD.

Our findings demonstrate that KIM-1 and TNFR1 levels are significantly elevated in diabetic patients, even in the absence of DKD, compared to controls, and escalate further with DKD. This suggests potential renal dysfunction before conventional signs of renal disease appear.

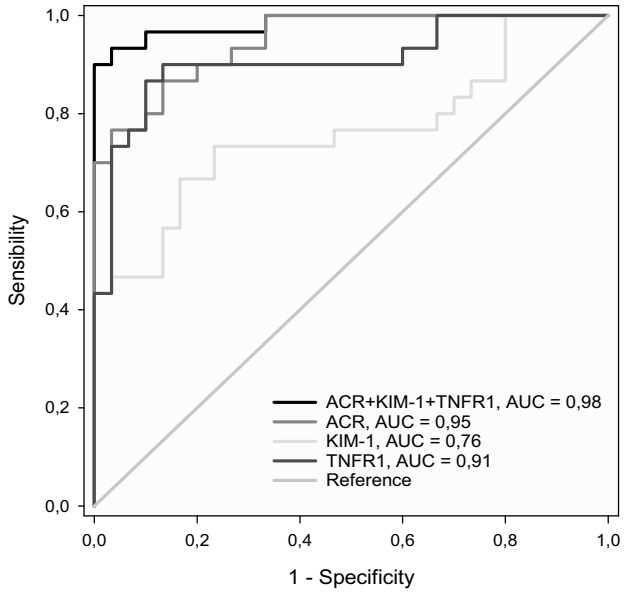
Elevated levels of KIM-1 and TNFR1 were observed in diabetic patients with normoalbuminuria compared to non-diabetic individuals, aligning with previous research [24, 25]. These findings suggest KIM-1 and TNFR1 as early indicators of DKD. Interestingly, KIM-1 levels did not significantly vary across different albuminuria stages, indicating its reflection of early tubular damage rather than overall kidney disease severity. Conversely, the marked increase in TNFR1 levels across albuminuria stages underlines its utility in monitoring renal disease progression. Significant increases in KIM-1 and TNFR1 were also observed with decreasing eGFR, with KIM-1 specifically rising in cases of moderate renal impairment ($30 \leq eGFR < 60$ ml/min/1.73m^2). However, the decrease in KIM-1 levels shown in patients with severe renal function impairment (eGFR < 30 ml/min/1.73m^2) is possibly due to diminished KIM-1 production by renal cells, as renal function is severely impaired in these cases. These observations imply that TNFR1 may serve as a superior marker for assessing kidney disease severity, whereas KIM-1 could a specific marker of moderate kidney impairment.

KIM-1 and TNFR1 levels correlated positively with the duration of diabetes and ACR, and negatively with eGFR. Additionally, TNFR1 showed a positive correlation with age, while no significant correlations were found between KIM-1 and TNFR1 with BMI and HbA1c, consistent with other studies [12, 13]. A positive correlation between KIM-1 and TNFR1 has been reported, with TNFR1 linked to increased inflammatory cytokine production via the NF-kB signaling pathway in chronic hyperglycemia [23], potentially

Table 4 Logistic regression analysis of kidney disease risk

	KIM-1		TNFR1		ACR	
	OR(IC, 95%)	***p***	**OR(IC, 95%)**	***p***	**OR(IC, 95%)**	***p***
Crude	1.015(1.005 – 1.026)	0.004	1.003(1.001 – 1.005)	0.000	1.179(1.066 – 1.304)	0.000
Model 1	1.015(1.004 – 1.027)	0.007	1.003(1.001 – 1.005)	0.000	1.169(1.050 – 1.301)	0.004
Model 2	1.017(1.005 – 1.030)	0.007	1.003(1.001 – 1.005)	0.000	1.178(1.047 – 1.326)	0.007
Model 3	1.021(1.006 – 1.037)	0.006	1.004(1.001 – 1.006)	0.003	1.182(1.035 – 1.349)	0.013

Model 1, adjusted for age, sex and BMI; Model 2, adjusted for all variables in model 1 + duration of diabetes and hypertension; Model 3, adjusted for all variables in model 2 + HDL-c. OR, odds ratio; CI, confidence interval; KIM-1, kidney injury molecule-1; TNFR1, tumor necrosis factor receptor1; ACR, albumin/creatinine ratio



	AUC(95%CI)	Cut-off(pg/ml)	Sensibility% (95%CI)	Specificity% (95%CI)
KIM-1	0.76(0.64-0.89)	71.13	73.33(54.11-87.72)	76.67(57.72-90.07)
TNFR1	0.91(0.83-0.99)	1868	90.00(73.47–97.89)	86.67(69.28-96.24)
ACR	0.95(0.90-1.00)	29.63	73.33(54.11–87.72)	96.67(82.78-99.92)
		17.42[#]	90.00(73.47–97.89)	80.00(61.43-92.29)
ACR+KIM-1+TNFR1	0.98(0.96-1.00)	-	96.67(82.78–99.92)	90.00(73.47–97.89)

Fig. 2 ROC curve AUC, area under the curve; ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; TNFR1, tumor necrosis factor receptor1; CI, confidence interval. [#]Optimal threshold value for ACR in the study

upregulating KIM-1 expression in renal epithelial cells under chronic inflammation [22].

Logistic regression confirmed a significant association between these markers and kidney disease, aligning with findings from larger diabetes cohorts [25], endorsing KIM-1 and TNFR1 as reliable predictors of kidney disease. ROC analysis revealed TNFR1's exceptional ability to discriminate between diabetic patients with and without DKD, comparable to that of ACR. The combination of TNFR1, KIM-1, and ACR enhanced diagnostic accuracy, supporting a multi-marker strategy [26, 27]. Moreover, we identified an ACR threshold of 17.42 mg/g, yielding 90% sensitivity and 80% specificity, an improvement over the traditional 30 mg/g threshold, which has been criticized for its lack of sensitivity in early DKD detection [28]. With the KDOQI recommendation of defining normal albuminuria as ACR < 10 mg/g (optimal) and 10 ≤ ACR < 30 mg/g (upper normal limit) [29] and other studies proposing different optimal ACR cut-off values [26], there's evidence that even a slight increase in baseline ACR within the normal range poses a risk for developing albuminuria [26, 30]. Research has shown that tubular markers, including KIM-1 and NGAL, are higher in T2D patients with ACR ranging from 10–30 mg/g compared to those with ACR < 10 mg/g [31]. Albuminuria indicates not only glomerular filtration damage but also contributes to tubulointerstitial injury through inflammation, oxidative stress, and fibrosis [26]. Thus, early intervention in albuminuria may prevent tubular damage and slow disease progression. Consequently, we suggest adjusting the albuminuria threshold to recognize 10–30 mg/g as indicative of increased kidney injury risk in T2D, potentially improving DKD risk identification, especially in resource-constrained settings.

It is important to note that our suggestion to reconsider the albuminuria threshold is based on observed trends, indicating a lower threshold might improve early DKD detection. However, the lack of subgroup analysis (e.g., DKD with and without albuminuria) and our small sample size limit the strength of this conclusion. While promising, our results should be interpreted with caution. Larger studies with detailed subgroup analyses are needed to validate the potential benefits of adjusting the albuminuria threshold for early DKD detection. Future research should focus on these aspects for more definitive evidence.

Study limitations and strengths

Our study's primary limitation is its case–control design and small sample size, which could compromise the generalizability and longitudinal monitoring capabilities of our findings. Moreover, accurately evaluating the relationship between DKD and KIM-1/TNFR1 levels in patients with normal albumin levels proved to be challenging. In future investigations, it would be beneficial to conduct multicentric studies across various geographical areas to confirm and extend our results. Despite these limitations, this study serves as a pioneering effort in examining early predictive markers for DKD in Benin, laying the groundwork for future research endeavors. Notably, our study's strength lies in employing minimally invasive measures for KIM-1, TNFR1, and ACR, enabling straightforward and repeat evaluations in high-risk individuals.

Conclusion

Our study suggests that both KIM-1 and TNFR1 hold promise as reliable predictors of DKD, with TNFR1 showing stronger discriminatory ability and closer ties to risk factors and disease severity. Combining TNFR1, KIM-1, and albuminuria could enhance the accuracy of DKD diagnosis. However, we recognize the limitations of our study, including the relatively small sample size and the lack of detailed subgroup analyses. Given these limitations, our findings regarding the albuminuria threshold should be considered preliminary. While our data indicate that an albuminuria threshold of 17.42 mg/g may enhance sensitivity and specificity for early DKD detection, this hypothesis requires validation through larger, multicentric studies with robust subgroup analyses. Further research is essential to confirm our results and to explore the potential of adjusting the albuminuria threshold to improve DKD risk identification, particularly in resource-constrained settings.

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Author contributions All authors contributed to the conception and design of the study. Material preparation, data collection and analysis were carried out by Carina P.A. ALOFA, Marcos A.D.F. MIGAN, Riel A.N. AMOUSSOU and Antoine FANDOHAN under the supervision of Patrice H. AVOGBE, Julien A.G. SEGBO and Casimir D. AKPOVI. The first draft of the manuscript was written by Carina P.A. ALOFA and Espérance F.E. KOUGNIMON and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability The data that support the findings of this study are available from the corresponding author upon request.

Declarations

Ethical approval The study was approved by the Local Ethics Committee for Biomedical Research (CLERB-UP), REF: 0580/CLERB-UP/P/SP/R/SA and carried out in accordance with relevant regulations.

Consent of patient The study was approved by the Local Ethics Committee for Biomedical Research. All patients of the study were under the care of a specialist and provided written consent after receiving clear explanations about the study objectives.

Conflict of interest The authors declare no conflict of interests.

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