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A combination of urinary neutrophil gelatinase-associated lipocalin and urine albumin creatinine ratio as potential biomarkers for the early diagnosis of CKD in type 2 diabetic patients

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KEYWORDS
Neutrophil gelatinase-associated lipocalin; Diabetes; Diabetic nephropathy.

ABSTRACT

One-third of patients with type 2 diabetes (T2DM) tend to develop chronic kidney disease (CKD) called diabetic nephropathy. The aim of this study was to investigate neutrophil gelatinase-associated lipocalin (NGAL) as a new potential biomarker to predict CKD in T2DM patients. A total of 93 participants were enrolled in the study and are divided into 3 groups including T2DM group (n = 16), T2DM with CKD group (n = 52) and healthy control group (n = 25). All the participants were tested for urinary NGAL (uNGAL) levels by enzyme-linked immunosorbent assay. The results revealed that the T2DM patients with CKD had a significantly higher level of uNGAL (21.0 ± 32.6 ng/mL) than the healthy controls (1.9 ± 2.0 ng/mL *p*-value = 0.005), and the uNGAL level in the T2DM patients with CKD was increased, but not significantly, compared to that in the T2DM patients without CKD (7.6 ± 6.4 ng/mL, *p*-value = 0.1067). Moreover, the analysis revealed moderate correlations between uNGAL and estimated glomerular filtration rate (eGFR) (r^2 = -0.450, *p*-value = 0.001) and between uNGAL and urine albumin creatinine ratio (ACR) (r^2 = 0.489, *p*-value = 0.000). The ROC curve analysis showed that uNGAL was more sensitive (69.23%) and specific (80.49%) than eGFR but could not reach the standard of ACR. Therefore, the study concludes that uNGAL can be a potential predictor of CKD in T2DM when combined with ACR.

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Introduction

Diabetes mellitus (DM) a major clinical and public health problem and affects approximately half a billion people worldwide; type 2 DM itself accounts for nearly 90% of the burden[1]. The condition is associated with several complications, such as cardiovascular disease, neuropathy, stroke and renal failure. A previous study showed that approximately one-third of diabetic patients develop diabetic nephropathy (DN)[2]. In Thailand, the 2015 renal replacement therapy report (TRT) revealed that 38.57% of end stage renal disease (ESRD) patients undergoing dialysis suffered from DN[3].

Glomerular damage is crucial for the initiation of DN due to continuous exposure to many metabolic and hemodynamic factors that lead to injury[4]. Renal tubule-interstitial fibrosis secondary to initial glomerular damage, however, is a potential factor of chronic kidney disease (CKD) progression in diabetes patients[5]. The early diagnosis of DN, as in other kidney diseases, is crucial for better treatment, preventing the progression to ESRD and thus increasing life expectancy and lowering health costs. Clinically, DN is diagnosed according to the guidelines of Kidney Disease Improving Global Outcomes (KDIGO), in which the estimated glomerular filtration rate (eGFR) derived from serum creatinine and daily urine albumin are used as standard kidney function markers[6]. However, an increase in serum creatinine above the physiological level is observed only when half of the kidney function is lost[7]. In other words, serum creatinine cannot accurately explain kidney health until a critical level of kidney function is reached. Urinary albumin is the gold standard for diagnosing and classifying the stages of DN[8] and accurately reflects the degree of glomerular damage; however, it is unable to fully reflect the degree of renal tubular injury[9]. Therefore, there is an immense requirement for a sensitive biomarker for the early diagnosis of DN as well as other causes of CKD, and neutrophil gelatinase-associated lipocalin (NGAL) is a potential candidate.

NGAL is a 25-kDa glycoprotein expressed in cells and tissues, such as neutrophils, monocytes, colon, stomach, trachea, lung, salivary glands, prostate, uterus, and kidney[10]. NGAL belongs to the lipocalin family, a protein family that transports small hydrophobic molecules (*e.g.*, steroids and lipids) and is associated with inflammation, transportation of pheromones, and synthesis of prostaglandins[11]. The levels of NGAL increase in both serum and urine within two hours upon acute tubulointerstitial injury, as NGAL is highly expressed by tubular renal epithelial cells, and indicates acute renal injury before the rise in serum creatinine concentrations[11,12]. Additionally, both serum NGAL and urine NGAL (uNGAL) were also related to tubule-interstitial fibrosis in various CKD models[13]. In a study carried out among patients with DN, an elevated uNGAL level was reported to be associated with the progressive course of the disease leading to ESRD[14]. Similarly, an observational follow-up study revealed that high uNGAL levels at baseline correlated with a rapid decline in eGFR levels in T2DM patients[15]. Therefore, NGAL can be a promising novel biomarker for the early diagnosis of tubular damage.

The current study aimed to investigate uNGAL as a potential marker for CKD in diabetic patients. The levels of uNGAL were analyzed and correlated with the standard biomarkers of kidney function, eGFR and urine albumin creatinine ratio (ACR), in T2DM patients with CKD, T2DM patients without CKD and healthy controls to evaluate the most appropriate biomarkers for the detection of CKD occurrence and severity in T2DM patients.

Materials and methods

Study design and population

This cross-sectional study was conducted among 93 participants grouped into healthy controls (n = 25), T2DM patients without CKD (n = 16) and T2DM patients with CKD (n = 52). The control group was composed of 25 volunteers who were undergoing an annual health checkup without a history of hypertension, diabetes, cancer, and diseases of the cardiovascular,

inflammatory, renal, lung and endocrine systems and who were not receiving any medical treatment. The diabetic participants had been cared for one year or longer at the Udon Thani Hospital, which provides tertiary care and is in the northeast of Thailand approximately 550 km from the capital city. Diabetic patients who had leukocytosis, i.e., white blood cell count > 10,000 cells/μL, and incomplete data for analyses were excluded. The study was approved by the Medical Ethics Committee of Khon Kaen University (HE621170) and conducted between January and December 2019.

A total of 68 T2DM patients were then categorized into two groups, i.e., with and without CKD based on their previous eGFR and urine ACR. CKD was defined according to the guidelines of Kidney Disease Improving Global Outcomes (KDIGO), indicating the presence of kidney damage and/or reduction in estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 for 3 months or longer[6]. In brief, T2DM patients with ACR < 30 mg/g and eGFR > 60 mL/min/1.73 m^2 were categorized into the T2DM without CKD group (n = 16) and patients with ACR > 30 mg/g or eGFR < 60 mL/min/1.73 m^2 ≥ 3 months into the T2DM with CKD group (n = 52). The T2DM with CKD group was further categorized into five subgroups based on eGFR as CKD stage 1 (eGFR ≥ 90 mL/min/1.732 m^2), CKD stage 2 (eGFR 60-89 mL/min/1.732 m^2), CKD stage 3 (eGFR 30-59 mL/min/1.732 m^2), CKD stage 4 (eGFR 15-59 mL/min/1.732 m^2), and CKD stage 5 (eGFR < 15 mL/min/1.732 m^2). Additionally, albuminuria was classified as A1 (normal to mildly increased, ACR < 30 mg/g), A2 (moderately increased, ACR = 30-300 mg/g), and A3 (severely increased, ACR > 300 mg/g).

Collection and preparation of samples

The participants' information on sex, age, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI) and diabetes duration were recorded with their consent. Fasting blood was collected by performing venipuncture followed by centrifugation at 3,500 rpm for 10 min at room temperature, and approximately 10 mL midstream urine was collected in a plastic container. The samples were analyzed immediately or aliquoted into sterile tubes and stored at -80 °C until analysis.

Sample analysis

The samples were analyzed for fasting blood sugar (FBS), hemoglobin A_1C (HbA_1C), serum creatinine (SCr), estimated glomerular filtration rate (eGFR), cholesterol (CHO), triglyceride (Tri), urine creatinine (UCr), urine microalbumin (UAlb), urine albumin/creatinine ratio (ACR), and uNGAL. The levels of SCr, FBS, and UAlb were measured by a fully automated chemiluminescent ARCHITECT platform (Abbott Diagnostics, Lake Forest, IL, USA), whereas HbA_1C was quantified by a G8 automated high-performance liquid chromatography (HPLC) glycohemoglobin analyzer (HLC-723G8 HPLC analyzer, Tosoh Co., Tokyo, Japan). uNGAL was measured by using an enzyme-linked immunosorbent assay (ELISA) kit (Millipore, Saint Louis, MO, USA) in 96-well plates coated with anti-human lipocalin-2. According to the protocol from the manufacturer, 100 μL of diluted NGAL-calibrator (range 4-1000 pg/mL) and diluted urine samples were added to the respective microwells followed by incubation at room temperature for 2.5 hours. Next, the wells were washed, and a biotinylated detection antibody specific for the target protein was added. After washing away unbound biotinylated antibody, horseradish peroxidase (HRP)-conjugated streptavidin was pipetted into the wells. The wells were washed again, and tetramethylbenzidine (TMB) substrate solution was added. Finally, the absorbance of the solution was measured at 450 nm [16]. All the standards and urine samples were tested in triplicate.

Statistical analysis

Statistical analysis of the data was performed using STATA version 14.2 (StataCorp, Texas, USA) and GraphPad Prism v.6 (GraphPad Software, Inc., La Jolla, CA, USA) software. Continuous variables were reported as means ± standard deviation or medians (minimum-maximum). The differences of all groups were established by one-way ANOVA or Kruskal-

Wallis test where appropriate. The differences between two groups were analyzed by unpair t-test as parametric test or Mann-Whitney U tests for nonparametric variables. The correlation was calculated using the Spearman test. A *p*-value of < 0.05 was considered statistically significant. The cut-off point, sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), and area under-ROC curves were also determined.

Results

Demographic and clinical data

The detailed demographic data and laboratory findings of the three groups are shown in Table 1. The average age of the patients in the T2DM with CKD group (59 ± 13) was higher than that in the T2DM without CKD (53 ± 12) and control (49 ± 11) groups. On the other hand, the eGFR was lower in the T2DM with CKD group (73.2 ± 32.6) than in the T2DM without CKD group (95.0 ± 19.4) and the healthy control group (99.4 ± 6.4). The ACRs in the T2DM with CKD, T2DM without CKD and control groups were 931.3 ± 2300, 13.7 ± 7.8 and 13.3 ± 8.38 mg/g Cr, respectively. The levels of uNGAL were significantly different among the three groups (*p*-value < 0.001, the *post hoc test* revealed *p*-value = 0.01 for control vs. DM without CKD groups, *p*-value < 0.001 for control vs. DM with CKD groups and *p*-value = 0.02 for DM without CKD vs. DM with CKD). The uNGAL level was approximately three times higher in the T2DM with CKD group (21.0 ± 32.6) than in the T2DM without CKD group (7.6 ± 6.4).

Table 2 shows the demographic and clinical data of the diabetic patients with various stages of CKD. The highest number of patients in the T2DM with CKD group was in stage I (42%), and the lowest average age was also in this group (50 ± 10). Levels of uNGAL gradually increased with the stages of CKD; the uNGAL levels of patients with CKD stages 1 and 2 were significantly lower than those of patients with CKD stages 4 and 5 (all *p*-value < 0.05) and significantly lower than those of patients with CKD stage 3 (*p*-value = 0.054).

Table 1 Demographic and clinical data of healthy control, T2DM without CKD and T2DM with CKD groups

Characteristic /Marker	Healthy control (n = 25)	T2DM without CKD (n = 16)	T2DM with CKD (n = 52)
Sex (Male/Female)	11/14	7/9	23/29
Age (years)*	49 ± 11	53 ± 12	59 ± 13
Systolic BP (mmHg)*	119 ± 12	132 ± 23	139 ± 17
Diastolic BP (mmHg)	76 ± 7	76 ± 15	77 ± 12
BMI (Kg/m^2)*	23.7 ± 3.3	26.3 ± 4.4	25.9 ± 4.0
Diabetes duration (years)	-	3 (range1-8)	3 (range1-8)
FBS (mg/dL)*	89 ± 9.1	188 ± 90	191 ± 76
HbA1C (%)*	5.4 ± 0.5	9.2 ± 2.4	9.4 ± 2.1
SCr (mg/dL)*	0.8 ± 0.2	0.79 ± 0.17	1.24 ± 0.96
eGFR (mL/min1.73/ m^2)*	99.4 ± 6.4	95.0 ± 19.4	73.2 ± 32.6
CHO (mg/dL)	192 ± 18	181 ± 58	203 ± 85
Tri (mg/dL)*	130 ± 46	122 ± 44	220 ± 146
UAlb (mg/dL)*	8.9 ± 4.2	16 ± 12	812 ± 1798
UCr (mg/dL)	101.0 ± 84.4	140.4 ± 90.3	108.3 ± 68.2
ACR (mg/g Cr)*	13.3 ± 8.38	13.7 ± 7.8	931.3 ± 2300
uNGAL (ng/mL), mean ± SD*	1.9 ± 2.0	7.6 ± 6.4	21.0 ± 32.6
median (min-max)*	0.9 (0-7.7)	5.1 (0.9-22.1)	8.3 (1.2-146.9)

Note: * p-value < 0.05 compared among three groups. ACR, albumin creatinine ratio; BP, blood pressure; BMI, body mass index; CHO, cholesterol; eGFR, glomerular filtration rate; FBS, fasting blood sugar; HbA1C, hemoglobin A1C; Kg, kilogram; Tri, triglyceride; SCr, serum creatinine; UAlb, urine albumin; UCr, urine creatinine; uNGAL, urinary neutrophil gelatinase-associated lipocalin; SD, standard deviation; min, minimum; max, maximum.

Table 2 Demographic and clinical data of diabetic patients with various CKD stages

Characteristic /Marker	T2DM with CKD				
	Stage1 (n =22)	Stage2 (n = 10)	Stage3 (n =13)	Stage4 (n = 5)	Stage5 (n = 2)
Sex (Male/Female)	11/11	3/7	5/8	3/2	1/1
Age (years)*	50 ± 10	63 ± 10	67 ± 13	65 ± 6	65 ± 18
Systolic BP (mmHg)	139 ± 16	142 ± 11	134 ± 17	143 ± 28	169 ± 11
Diastolic BP (mmHg)	82 ± 12	74 ± 11	74 ± 10	77 ± 16	74 ± 17
BMI (Kg/m^2)	26.7 ± 4.3	24.8 ± 4.0	26.3 ± 3.9	25.6 ± 3.5	22.0 ± 2.4
Diabetes duration (years)	4 (1-8)	3 (1-6)	3 (2-6)	2 (1-3)	3 (2-4)
FBS (mg/dL)	200 ± 78	169 ± 46	188 ± 67	165 ± 41	291 ± 231
HbA1C (%)	10.0 ± 2.3	9.0 ± 1.6	9.2 ± 1.9	7.7 ± 0.6	10 ± 5.4
SCr (mg/dL)*	0.7 ± 0.2	0.9 ± 0.1	1.3 ± 0.29	2.6 ± 1.0	4.6 ± 1.2
Egfr (mL/min1.73/ m^2)*	104.9 ± 12.6	73.0 ± 7.6	48.2 ± 5.3	24 ± 6.7	11.5 ± 4.3
CHO (mg/dL)*	206 ± 56	178 ± 60	211 ± 118	184 ± 62	401 ± 383
Tri (mg/dL)	226 ± 162	161 ± 89	244 ± 149	189 ± 79	326 ± 148
UAlb (mg/dL)*	583.3 ± 1440.7	164 ± 141	1142 ± 1598	594 ± 361	4970 ± 6949
UCr (mg/dL)	80.9 ± 42.1	135.3 ± 94.1	143.0 ± 76.4	94.3 ± 44.2	83.3 ± 12.3
ACR (mg/g)*	814.5 ± 2024.6	145.3 ± 156.3	918 ± 1393	762.6 ± 594	6651 ± 9320
uNGAL (ng/mL), mean ± SD*	9.2 ± 6.6	12.4 ± 18.6	29.5 ± 42.7	47.0 ± 37.5	73.2 ± 95.4
median (min-max)*	7.6 (1.2-26.2)	5.6 (3.0-64.5)	9.6 (1.3-146.9)	36.8 (8.4-91.7)	73.2 (5.7-140.7)

Note: * *p*-value < 0.05 compared among five groups. ACR, albumin creatinine ratio; BP, blood pressure; BMI, body mass index; CHO, cholesterol; eGFR, glomerular filtration rate; FBS, fasting blood sugar; HbA1C, hemoglobin A1C; Kg, kilogram; Tri, triglyceride; SCr, serum creatinine; UAlb, urine albumin; UCr, urine creatinine; uNGAL, urinary neutrophil gelatinase-associated lipocalin; SD, standard deviation; min, minimum; max, maximum.

The distribution of albuminuria severity in the T2DM with CKD group was 9.6%, 53.9% and 36.5% for the A1, A2 and A3 categories, respectively. The mean and median uNGAL levels in the A1 group (n = 5) were 5.9 ± 5.3 and 2.9 (1.3-14), A2 group (n = 28) were 10.9 ± 13.7 and 6.4 (1.2-70.5) and A3 group (n = 19) were 39.9 ± 46.0 and 17.1 (1.6-146.9) ng/mL. The uNGAL level in the A3 group was higher than that in the A1 and A2 groups, with statistically significant *p*-value of 0.005 and 0.015, respectively (Figure 1).

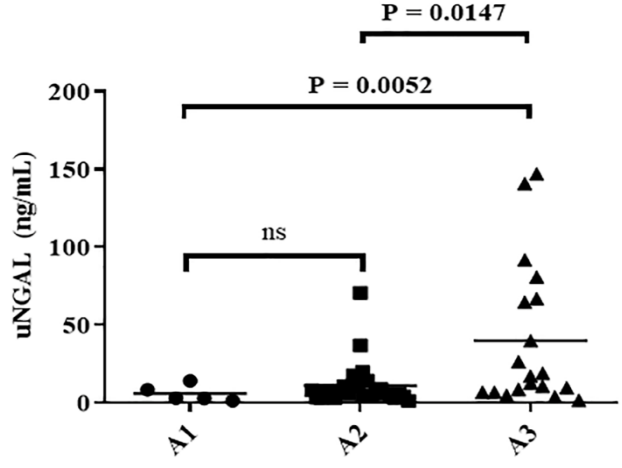

Figure 1 The comparison of uNGAL (mean ± SD) among various stages of albuminuria in T2DM with CKD subgroups.

Correlation between the uNGAL and eGFR/ACR levels in all participants

The association of the uNGAL levels with clinical parameters (eGFR or ACR) in all 93 participants is shown in Figure 2A and 2B. The analysis revealed moderate correlations between uNGAL and eGFR (r = -0.51, *p*-value < 0.001) and between uNGAL and ACR (r = 0.53, *p*-value < 0.001).

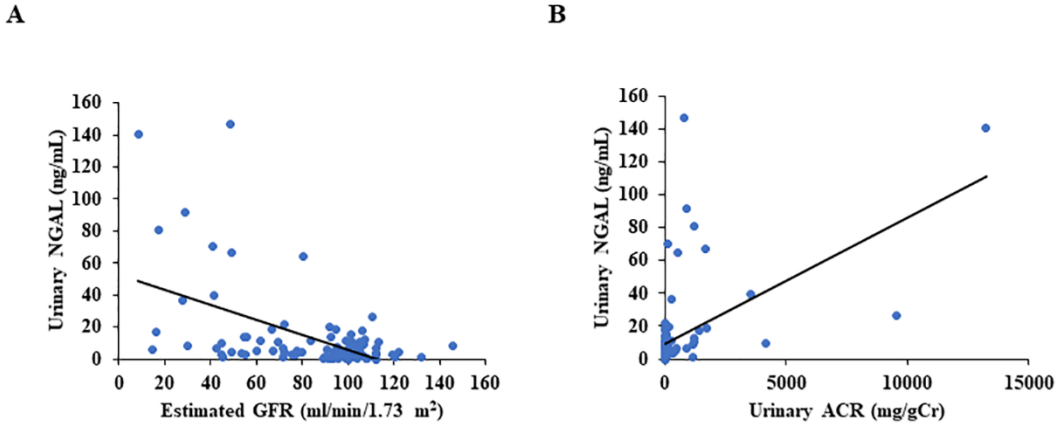

Figure 2 The correlation between (A) uNGAL and eGFR and (B) uNGAL and ACR in all participants.

Comparison of uNGAL, ACR, and eGFR biomarkers in the diagnosis of T2DM with CKD

ROC curve analysis was performed to elucidate whether the uNGAL, ACR and eGFR levels can be used to distinguish the T2DM with CKD group from the T2DM without CKD and healthy control groups (Figure 3A). The results showed that the AUC for uNGAL was 0.811; standard error (SE) = 0.045; 95% confidence interval = 0.722–0.899 with cut-off uNGAL level at ≥ 5.7 ng/mL and sensitivity = 69.23%, specificity = 80.49%, positive predictive value = 81.8% and negative predictive value = 67.3% (see Supporting Information, Table S1). The AUC for ACR was 0.952; SE = 0.022; 95% CI = 0.908–0.994. The AUC for eGFR was 0.281; SE = 0.053; 95% CI = 0.175–0.387 lower sensitivity and specificity than uNGAL.

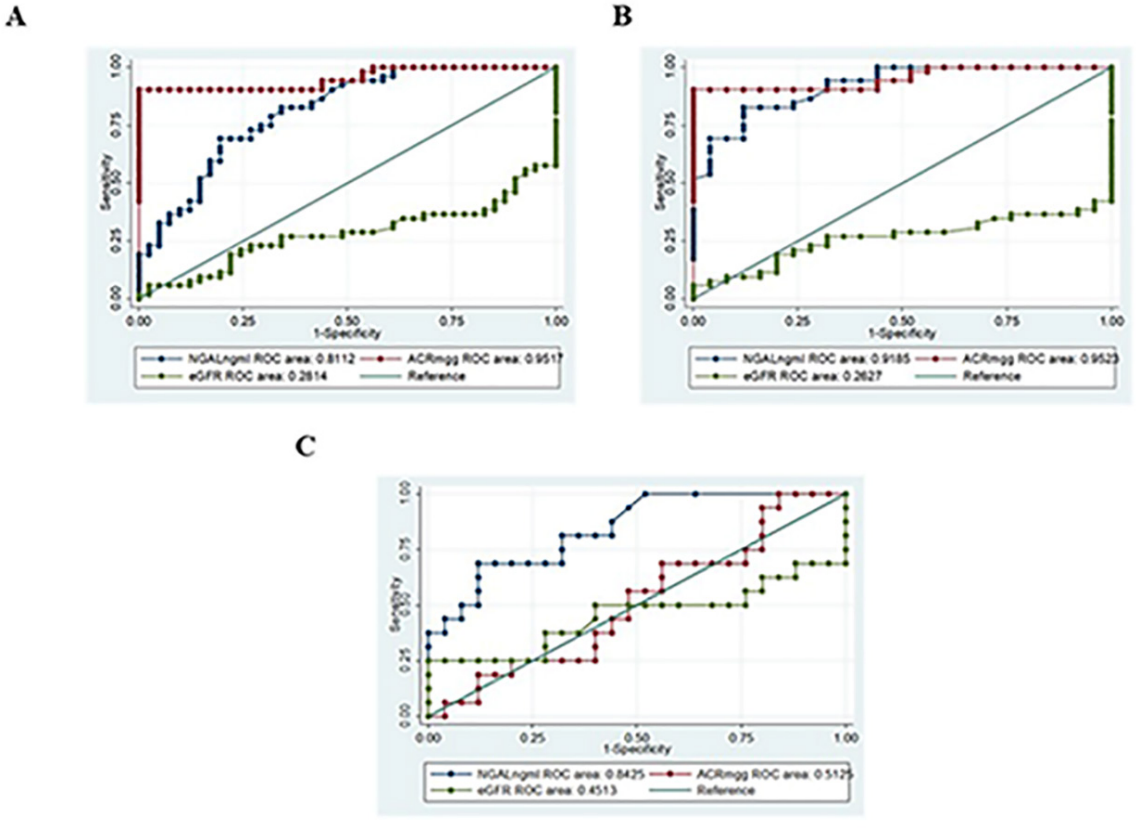

Figure 3 ROC curves of uNGAL, ACR, and eGFR (A) among the healthy control, T2DM without CKD and T2DM with CKD groups, (B) between the healthy control and T2DM with CKD groups, (C) between the healthy control and T2DM without CKD groups.

We further compared the ROC curves of uNGAL, ACR and eGFR between the healthy control and T2DM with CKD groups (Figure 3B). The AUC for uNGAL was 0.919; SE = 0.030; 95% CI = 0.857–0.979, which was comparable to the AUC for ACR (0.952; SE = 0.022; 95% CI = 0.908–0.996). The AUC for eGFR was 0.263; SE = 0.055; 95% CI = 0.153–0.372.

The comparison of the uNGAL, ACR and eGFR ROC curves between the healthy control and T2DM without CKD groups is shown in Figure 3C. The AUC for uNGAL was highest (0.842; SE = 0.060; 95% CI = 0.723–0.961). The AUC for ACR was 0.512; SE = 0.093; 95% CI = 0.328–0.696, and the AUC for eGFR was 0.451; SE = 0.109; 95% CI = 0.237–0.665.

ROC analyses of uNGAL levels in the diagnosis of various stages of T2DM with CKD

ROC analyses of uNGAL in T2DM patients with CKD (categorized according to the levels of albuminuria) demonstrated AUC (95% CI) as 0.69 (0.35-1.00) for A1 vs. A2, 0.83 (0.63-1.00) for A1 vs. A3 and 0.75 (0.48-1.00) for A1 vs A2 plus A3 (see Supporting Information, Figure S1).

Discussion

Previous studies clearly revealed that NGAL is a novel biomarker of CKD progression[13,18]. The predictive data of eGFR suggests already impaired renal function and it is an important marker for kidney damage progression. Both uNGAL and sNGAL represented a most powerful predictor even compared to adjustment of eGFR; therefore NGAL could predict CKD progression beyond the eGFR information[13]. Tubular injury may lead to glomerular damage in diabetic patients and NGAL is able to use as specific biomarker to predict DN even earlier to incipient nephropathy. Both sNGAL and uNGAL are noninvasive tools for predicting albuminuria and for diagnosis, staging, and monitoring of DN progression[17]. We proposed uNGAL as a potential biomarker of CKD progression in T2DM patients and compared it with classical kidney function markers, such as ACR and eGFR, in healthy controls and T2DM patients with or without CKD. The uNGAL level in T2DM patients with CKD was significantly higher than that in T2DM patients without CKD and normal healthy controls. Moreover, the uNGAL levels corroborated the severity of CKD based on albuminuria and eGFR in this study. A typical parallel correlation between increased uNGAL and albuminuria was observed among the patients, but the trends of uNGAL and eGFR were opposite. Although the absolute numbers may differ, these findings are similar in pattern to previous studies[17-19]. For example, a study conducted in Malaysia reported the average uNGAL levels in control subjects as 4.75 (0.1–27.5) ng/mL, the uNGAL levels in normoalbuminuria and microalbuminuria patients as 19.05 (range 1.1–60) ng/mL and 26.9 (range 3.7–603.5) ng/mL, respectively, and the uNGAL levels in DN patients as 28.55 (range 0.7–1500) ng/mL[20]. Our ROC curve analysis of uNGAL, ACR and eGFR was able to distinguish the T2DM with CKD group from the T2DM without CKD and healthy control groups. uNGAL as a marker was more sensitive (69.23%) and specific (80.49%) than eGFR but could not reach the standard of ACR.

NGAL is highly expressed in tubular renal epithelial cells following tubulointerstitial injury and is reflected in serum and urine within two hours[21] compared to serum creatinine, which is elevated after 24 hours of reperfusion[22]. Although NGAL was initially detected in acute kidney injury (AKI), it has now been used for evaluating CKD patients[23]. As such, findings suggest that uNGAL may represent a real-time indicator of renal damage and an independent predictor of renal disease progression in specific kidney diseases, such as glomerulonephritides[19]. uNGAL has also been shown to be an early biomarker for the degree of chronic tubule-interstitial injury in patients with IgA nephropathy[24]. Of importance, NGAL might also be elevated in other conditions, not necessarily pertaining only to renal injury[15]. Therefore, we excluded patients with infection, neoplasia, and inflammation from the study.

Herein, we found that some of the patients had normal albuminuria, but the uNGAL level was already significantly increased compared to that in the normal healthy controls. Our results corroborate earlier published reports[25,26]. This suggested that some kidney injury may have already occurred in the patients, which can be detected by measuring uNGAL but not albuminuria. The NGAL level is also known to increase in acute and severe pancreatitis in animal studies[27]. A high level of NGAL in pancreatic fluid was reported in a study in humans with chronic pancreatitis[28]. Therefore, there is an equal possibility that high uNGAL in diabetic patients may be due to pancreatic injury, inflammation or even silent kidney disease. NGAL is an acute phase protein, similar to C-reactive protein, that is produced during inflammation but mainly with transportation and bacteriostatic function.

A correlation between uNGAL and hyperlipidemia was revealed in the study, which may be related to metabolic syndrome. It has

been suggested that NGAL plays a significant role in both glucose and lipid metabolism[29]. Moreover, the NGAL concentration is reported to correlate linearly with obesity, hyperglycemia, and hyperglycemia in patients with metabolic and cardiovascular diseases[30]. An association between uNGAL and lipidemia in the study patients may thus be linked to the fact that NGAL antagonizes the insulin resistance-enhancing effects of tumor necrosis factor α in adipocytes and macrophages to protect against inflammation.

Previously, urinary and serum NGAL were reported to predict CKD progression independent of age and GFR[13]. A published report mentioned that the increase in NGAL in CKD is the consequence of sustained production by inflamed tubular cells, whereas the contraction of GFR is the mere passive result of a general loss of functional cells or nephrons. Importantly, our finding has implications for using uNGAL as a marker of CKD progression in patients with normal albuminuria or microalbuminuria when eGFR is still preserved above 60 mL/min/1.73 m^2. Finally, the present study has some limitations. This was a single-center study with relatively small numbers of patients. Additionally, the design of this research was cross-sectional, which limited the observations on change over time. A multicenter follow-up study may be essential for more detailed and precise findings.

Conclusion

The present study points out that uNGAL was more sensitive as a biomarker to predict CKD progression in T2DM patients compared to than eGFR but less profound than ACR. Our findings can be vital in an early diagnosis of CKD advancement especially in patients with preserved eGFR and normal or microalbuminuria. It can thus aid in timely treatment and proper management behavior to prevent CKD progression to ESRD.

Take home messages

The present study points out that uNGAL was more sensitive as a biomarker to predict CKD progression in T2DM patients compared to eGFR but less profound than ACR. Our findings can be vital in an early diagnosis of CKD advancement especially in patients with preserved eGFR and normal or micro albuminuria.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Supplementary

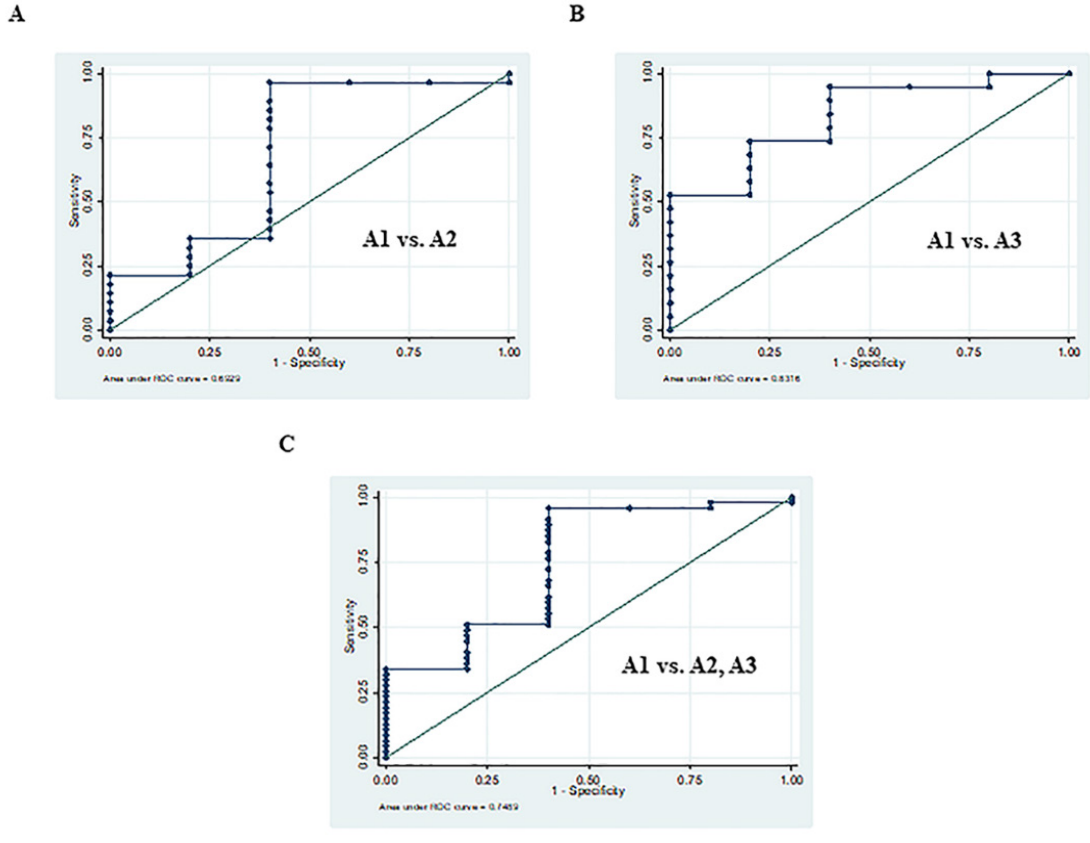

Figure S1 ROC analyses of uNGAL levels in the diagnosis of various stages of T2DM with CKD. (A) A1 vs. A2. (B) A1 vs. A3. (C) A1 vs. A2 and A3.

Table S1 Cut off uNGAL values between normal plus DM non-CKD and DM with CKD

uNGALvalue (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
≤5.7	69.23	80.49	81.8	67.3	3.54	0.38

Note: PPV, positive predictive value; NPV, Negative predictive value; +LR, Positive likelihood ratio; -LR, negative likelihood ratio.