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Original Article

Usefulness of the neutrophil gelatinase-associated lipocalin/inflammation index ratio for assessing diabetic nephropathy in patients with type 2 diabetes

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Abstract: Kidney function and inflammatory conditions can affect plasma neutrophil gelatinase-associated lipocalin (NGAL) levels. This study aimed to investigate the usefulness of the NGAL-to-inflammation index ratio (NGAL/Inf ratio) for assessing diabetic nephropathy in patients with type 2 diabetes mellitus (T2DM). A total of 195 patients with T2DM were evaluated by measuring plasma NGAL levels, the NGAL/Inf ratio, urine albumin excretion (UAE), glycemic parameters, and inflammatory parameters. The concentration of plasma NGAL was significantly higher in patients with T2DM than in healthy individuals (130.5 ng/mL versus 71.0 ng/mL, P < 0.001). The NGAL/Inf ratio of patients with microalbuminuria was greater than that of patients with normoalbuminuria. UAE was more closely associated with the NGAL/Inf ratio than plasma NGAL. After adjusting for potential confounders, an elevated NGAL/Inf ratio was significantly associated with an increased risk for microalbuminuria (odds ratio = 1.34, 95% CI = 1.09-2.54, P = 0.012). In comparison with plasma NGAL (area under the curve = 0.599, 95% CI = 0.517-0.681), the NGAL/Inf ratio (area under the curve = 0.742, 95% CI = 0.671-0.814) demonstrated significantly better diagnostic efficacy for identifying microalbuminuria (P < 0.001). Moreover, the NGAL/Inf ratio significantly improved risk prediction for diabetic nephropathy compared with predictions made using the NGAL. In conclusion, the corrected plasma NGAL level (NGAL/Inf ratio) could provide more accurate information than the uncorrected plasma NGAL level for assessing diabetic nephropathy in patients with T2DM.

Keywords: Neutrophil gelatinase-associated lipocalin, diabetic nephropathy, inflammation, microalbuminuria

Introduction

Diabetic nephropathy is a microvascular complication that occurs in approximately 40% of patients with type 2 diabetes mellitus (T2DM), which is characterized by albuminuria and loss of kidney function [1, 2]. Chronic hyperglycemia increases the production of oxidative free radicals and leads to inflammation and tubulointerstitial damage, which plays a major role in the development of diabetic nephropathy [3, 4]. There have been several attempts to identify an ideal biomarker that can detect the early stage of diabetic nephropathy and ultimately prevent the progression of disease.

A study has shown that neutrophil gelatinase-associated lipocalin (NGAL) can predict albuminuria in T2DM and can be used as an early biomarker of diabetic nephropathy [5]. However, there are conflicting results on the clinical usefulness of plasma NGAL in diabetic patients. In some studies, NGAL measurements were useful for evaluating renal dysfunction in diabetic patients and for diagnosing incipient nephropathy [6, 7]. However, in another study, NGAL measurements were not useful for discriminating diabetic patients with microalbuminuria from those with normoalbuminuria [8].

NGAL is a 25 kDa glycoprotein belonging to the lipocalin protein family [9]. NGAL has been used as an early indicator of acute kidney injury because NGAL rapidly increases within 2 h of renal tubular damage and is increased before serum creatinine elevation by 48 to 72 h [10].

However, a critical limitation of NGAL is that it increases in response to various inflammatory conditions [11]. Therefore, it is difficult to interpret the meaning of elevated NGAL levels in cases of renal dysfunction and concurrent inflammation, which commonly occur in patients with T2DM.

There are limited studies on the corrected level of plasma NGAL for assessing diabetic nephropathy in T2DM. Therefore, this study investigated the effectiveness of the NGAL-to-inflammation index ratio (NGAL/Inf ratio) with the aim of minimizing the effect of inflammation on plasma NGAL levels in diabetic patients. Additionally, to determine whether inflammation or kidney function contributes more to NGAL elevation in people with diabetes, NGAL levels were examined after adjustment using the delta estimated glomerular filtration rate (δeGFR).

Materials and methods

Subjects

A total of 247 subjects aged 29-73 years, including patients with newly diagnosed T2DM (n = 195) and age-matched healthy individuals (n = 52), were evaluated. To examine the ability of the NGAL/Inf ratio to identify diabetic nephropathy, patients with microalbuminuria (n = 74) and those with normoalbuminuria (n = 121) were included. T2DM was diagnosed using the diagnostic criteria of the American Diabetes Association [12]. The following participants were excluded from the study: (a) those who had sepsis, systemic inflammatory response syndrome, or fever ≥ 38.0°C; (b) those with current use of medications or surgery; (c) those with anemia or during pregnancy; (d) those with fasting time < 8 h. Information on the status of cigarette smoking was obtained using a questionnaire. The study protocol was approved by the institutional review board of Inha University Hospital (approval number: 2020-12-004). This study was performed in accordance with the guidelines of the Helsinki Declaration. All blood samples were collected after a sufficient explanation of the study procedure.

Measurement of laboratory parameters

Blood samples were collected from subjects after at least 8 h of fasting. All samples were obtained prior to treatment. The concentration of plasma NGAL was measured by fluorescence immunoassay using the Triage NGAL Test kit (Alere Inc., San Diego, CA, USA). A medical decision point of 150 ng/mL was set for plasma NGAL [13]. Serum and urine creatinine (Cr), urine albumin excretion (UAE), and plasma glucose levels were analyzed with a chemical analyzer (Cobas 8000 C702; Roche, Mannheim, Germany). The fraction of glycated hemoglobin (HbA1c) was measured by high-performance liquid chromatography using the G8 Glycohemoglobin Analyzer (Tosoh Bioscience, Tokyo, Japan). High-sensitivity C-reactive protein (hsCRP) levels were determined by particle-enhanced immunonephelometry assay (Dade Behring, Inc., Deerfield, IL, USA). The erythrocyte sedimentation rate (ESR) was measured using the Westergren sedimentation technique with StaRRsed Auto-Compact (Mechatronics Manufacturing BV, Zwaag, The Netherlands). The corrected ESR (cESR) was calculated based on a normal hematocrit of 45% using the following equation: cESR (mm/h) = (patient's hematocrit/45) × ESR (mm/h). An elevated hsCRP and an elevated cESR were defined as ≥ 0.3 mg/dL and ≥ 15 mm/h, respectively, which were based on a 95% confidence interval for the hsCRP and cESR of healthy individuals. The albumin-to-creatinine ratio (ACR) was calculated using the following formula: ACR (μg/mg Cr) = urine albumin (μg/mL)/urine Cr level (mg/dL). Normoalbuminuria and microalbuminuria were defined as ACR < 30 μg/mg Cr and 30-300 μg/mg Cr, respectively [14]. The eGFR was calculated using the Modification of Diet in Renal Disease formula: eGFR = 186 × [serum Cr (mg/dL)]$^{-1.154}$ × [age (years)]$^{-0.203}$. A correction factor of 0.742 was used for women. An eGFR < 60 mL/min/1.73 m^2 was regarded as renal dysfunction [15].

Calculation of corrected NGAL levels

The NGAL/Inf ratio was calculated using the following formula: NGAL/Inf ratio = plasma NGAL level/inflammation index. The inflammation index was obtained using the scores of hsCRP and cESR as described previously [16]. The cutoff limit of the NGAL/Inf ratio for assessing diabetic nephropathy in patients with T2DM was defined as 132 ng/mL. The cutoff limit was based on the highest sensitivity and specificity for identifying microalbuminuria in receiver operating characteristic (ROC) curve

Table 1. Clinical and laboratory characteristics of the patient population

Parameters	Patients with diabetes (n = 195)	
	Frequency (n)	Proportion (%)
Age (years; median, range)	64 (29-73)	NA
Sex (male)	102	52.3
Duration of disease (years)	1.2 (0.5-2.3)	NA
Laboratory parameters		
Elevated NGAL	71	36.4
Renal dysfunction	42	21.5
Systemic inflammation	107	54.8
Microalbuminuria	74	37.9
Clinical parameters		
Overweight	72	36.9
Dyslipidemia	53	27.1
Hypertension	25	12.8
Smokers	47	24.1

NGAL: neutrophil gelatinase-associated lipocalin; eGFR: estimated glomerular filtration rate; hsCRP: high-sensitivity C-reactive protein; cESR: corrected erythrocyte sedimentation rate; NA: not applicable.

analysis. Additionally, to adjust plasma NGAL levels for kidney function, the δeGFR was used [17]. In brief, the δeGFR was calculated using the equation: δeGFR = (90 - patient's eGFR)/90. When patients had an eGFR ≥ 90 mL/min/1.73 m^2, the eGFR was set to 90 mL/min/1.73 m^2 to avoid obtaining a negative value. The eGFR-index was determined as 1 + δeGFR. Finally, the adjusted NGAL/eGFR-index was obtained using the following equation: NGAL/eGFR-index = plasma NGAL level/(1 + δeGFR). The percent difference between plasma NGAL level and the corrected NGAL level was calculated using the following formula: [(NGAL - corrected NGAL)/NGAL] × 100.

Categorization of patients

Patients were categorized into two groups according to UAE: patients with microalbuminuria (n = 74) and those with normoalbuminuria (n = 121). To evaluate the effect of inflammation and kidney function on plasma NGAL levels, patients were further stratified into two groups: patients without elevated hsCRP (n = 88) and patients without impaired kidney function (n = 153).

Statistical analysis

Student's t-test and Mann-Whitney U test were used to analyze the data between two groups. Data were expressed as mean ± standard deviation (SD) or median (interquartile range: IQR). Categorical variables were presented as frequency and proportion. The ROC curve was analyzed to determine the ability of NGAL and the NGAL/Inf ratio to predict diabetic nephropathy. The net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were calculated to quantify the improvement in area under the ROC curve (AUC). To investigate the relationship between NGAL, the NGAL/Inf ratio, glycemic parameters, and renal function, multivariate linear regression analysis was performed following adjustment for potential confounders, such as age, gender, body mass index (BMI), systolic blood pressure (SBP), total cholesterol, and smoking habit. The odds ratio of an elevated NGAL/Inf ratio (> 132 ng/mL) for microalbuminuria was examined by multivariate logistic regression analysis. Data were analyzed using SPSS software (IBM SPSS Statistics, Armonk, NY, USA), MedCalc statistical software (MedCalc Software Ltd., Ostend, Belgium), and R statistical software package (R Foundation for Statistical Computing, Vienna, Austria). A value of $P < 0.05$ was considered statistically significant.

Results

Clinical and laboratory characteristics of subjects

Of the 195 diabetic patients, 71 (36.4%) patients had an elevated NGAL level, 74 (37.9%) patients had microalbuminuria, and 42 (21.5%) patients exhibited renal dysfunction with an eGFR < 60 mL/min/1.73 m^2. In addition, 107 (54.8%) patients had systemic inflammation with an elevated hsCRP ≥ 0.3 mg/dL, and 72 (36.9%) patients were overweight. The average duration of disease was 1.2 years (**Table 1**).

NGAL and its corrected levels in diabetic patients

The concentration of plasma NGAL was significantly higher in patients with T2DM than in healthy individuals (130.5 ng/mL versus 71.0 ng/mL, P < 0.001) (**Figure 1**). Among patients

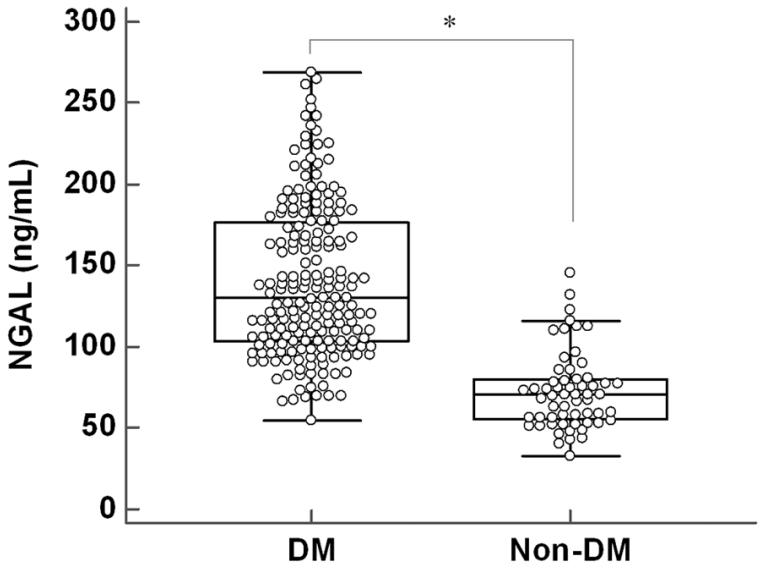

Figure 1. Plasma NGAL concentration of diabetic patients and healthy individuals. The concentration of NGAL is significantly higher in patients with DM than in non-DM healthy individuals (130.5 ng/mL versus 71.0 ng/mL) *P < 0.001.

with T2DM, the percent difference between NGAL and the NGAL/Inf ratio was 34.5%, which was significantly higher than that (13.4%) between NGAL and the NGAL/eGFR-index (P < 0.001) (**Table 2**).

NGAL/Inf ratio of patients with microalbuminuria

The NGAL/Inf ratio of patients with microalbuminuria was greater than that of patients with normoalbuminuria (98.3 ng/mL versus 71.2 ng/mL, P = 0.021). However, no significant difference in plasma NGAL and the NGAL/eGFR-index was observed between the two groups (**Table 3**).

Effect of inflammation and kidney function on NGAL

The effect of systemic inflammation and renal dysfunction on plasma NGAL levels was evaluated. After excluding patients with a decreased eGFR < 60 mL/min/1.73 m^2 from the sample population, plasma NGAL remained higher in diabetic patients than in healthy controls (112.5 ng/mL versus 71.0 ng/mL, P < 0.001). In contrast, after excluding patients with an elevated hsCRP ≥ 0.3 mg/dL from the sample population, plasma NGAL was not significantly different between the two groups (**Table 4**).

Relationship between NGAL/Inf ratio and renal function

After adjusting for potential confounders, serum creatinine and UAE were more closely associated with the NGAL/Inf ratio than plasma NGAL. Glycemic parameters showed no significant association with plasma NGAL and the NGAL/Inf ratio (**Table 5**). Scatter plots of the relationship between UAE and the NGAL/Inf ratio are shown in **Figure 2**.

NGAL/Inf ratio as a risk factor for microalbuminuria

Logistic regression analysis of risk factor for microalbuminuria was performed, and odds ratio of each parameter was summarized in **Table 6**. To investigate whether an elevated NGAL/Inf ratio is associated with the development of microalbuminuria, multivariate logistic regression analysis was conducted. An elevated NGAL/Inf ratio > 132 ng/mL was significantly associated with the prevalence of microalbuminuria in patients with diabetes (odds ratio = 1.34, 95% CI = 1.09-2.54, P = 0.012) (**Table 6**).

Diagnostic efficacy of NGAL and NGAL/Inf ratio

The ability of plasma NGAL and the NGAL/Inf ratio to identify microalbuminuria in patients with T2DM was assessed. ROC curve analysis revealed that the AUC of the NGAL/Inf ratio was significantly larger than that of plasma NGAL (0.742, 95% CI = 0.671-0.814 versus 0.599, 95% CI = 0.517-0.681, P < 0.001) (**Figure 3**). The NGAL/Inf ratio significantly improved risk prediction for diabetic nephropathy when evaluated using NRI (0.311; 95% CI, 0.025-0.642, P = 0.032) and IDI (0.057; 95% CI, 0.015-0.098, P = 0.016).

Discussion

This study showed that plasma NGAL levels were significantly higher in diabetic patients than in healthy individuals. Our results are in

Table 2. Corrected NGAL levels in patients with diabetes and healthy individuals

Parameters	Patients (n = 195)	Healthy individuals (n = 52)	*P* value
Anthropometric parameters			
Age (years)	64.8 ± 14.9	62.7 ± 12.4	0.351
Sex (male, n, %)	102 (52.3)	27 (51.9)	0.952
BMI (kg/m^2)	23.1 ± 3.5	21.4 ± 2.9	0.002
Corrected NGAL (ng/mL)			
NGAL/Inf ratio	85.3 (65.7-161.8)	64.0 (50.3-102.7)	0.034
NGAL/eGFR-index	114.2 (76.5-240.4)	68.1 (52.4-108.5)	< 0.001
Percent difference (%)			
NGAL versus NGAL/Inf ratio	34.5 ± 21.8*	8.3 ± 6.4	< 0.001
NGAL versus NGAL/eGFR-index	13.4 ± 10.9	3.6 ± 2.5	< 0.001
Glycemic parameters			
Fasting plasma glucose (mmol/L)	9.1 ± 2.5	5.5 ± 0.4	< 0.001
HbA1c (%)	6.8 ± 1.3	5.6 ± 0.3	< 0.001
Kidney function			
Serum creatinine (mg/dL)	1.15 ± 0.47	0.81 ± 0.23	< 0.001
eGFR (mL/min/1.73 m^2)	84.3 ± 16.2	93.2 ± 7.5	< 0.001
Inflammatory parameters			
HsCRP (mg/dL)	0.52 (0.17-2.93)	0.08 (0.06-0.25)	< 0.001
cESR (mm/h)	18.1 ± 12.4	7.6 ± 5.1	< 0.001

Data are expressed as mean ± SD, median (IQR), or frequency (%). *Significant (P < 0.001), compared with the percent difference between NGAL and the NGAL/eGFR-index. BMI: body mass index; NGAL: neutrophil gelatinase-associated lipocalin; NGAL/Inf ratio: NGAL-to-inflammation index ratio; HbA1c: glycated hemoglobin; eGFR: estimated glomerular filtration rate; hsCRP: high-sensitivity C-reactive protein; cESR: corrected erythrocyte sedimentation rate.

Table 3. Plasma NGAL and its corrected levels in patients with microalbuminuria and normoalbuminuria

Parameters	Microalbuminuria (n = 74)	Normoalbuminuria (n = 121)	*P* value
Anthropometric parameters			
Age (years)	65.3 ± 12.5	63.9 ± 13.2	0.463
Sex (male, n, %)	39 (52.7)	63 (52.0)	0.921
BMI (kg/m^2)	22.3 ± 3.2	23.2 ± 4.1	0.108
Lipocalin (ng/mL)			
Plasma NGAL	151.6 (85.0-257.5)	123.4 (83.0-249.5)	0.085
NGAL/Inf ratio	98.3 (67.6-161.2)	71.2 (52.3-125.4)	0.021
NGAL/eGFR-index	120.4 (81.2-206.7)	109.6 (74.1-217.3)	0.324
Albuminuria			
UAE (μg/mL)	76.1 ± 38.5	22.8 ± 5.2	< 0.001
ACR (μg/mg Cr)	53.2 ± 21.6	13.0 ± 4.7	< 0.001

Data are expressed as mean ± SD, median (IQR), or frequency (%). BMI: body mass index; NGAL: neutrophil gelatinase-associated lipocalin; NGAL/Inf ratio: NGAL-to-inflammation index ratio; UAE: urine albumin excretion; ACR: albumin-to-creatinine ratio.

agreement with the results of a previous study, which demonstrated that the concentration of serum NGAL was significantly increased in diabetics compared with non-diabetics [18]. However, T2DM is commonly accompanied by systemic inflammation; thus, the level of plasma NGAL is prone to overestimation [19]. In our study, more than half of the diabetic patients had an elevated hsCRP concentration. Therefore, the elevated NGAL observed in our patients may be attributed to inflammation. Under these conditions, the level of plasma

Table 4. Plasma NGAL concentration after excluding diabetic patients with inflammation and renal dysfunction

Parameters	Patients with diabetes (n = 195)		Healthy individuals (n = 52)
	After excluding inflammation (n = 88)	After excluding renal dysfunction (n = 153)	
Age (years)	63.9 ± 14.2	64.1 ± 13.5	62.7 ± 12.4
Sex (male, n, %)	42 (47.7)	75 (49.0)	27 (51.9)
Plasma NGAL (ng/mL)	74.0 (50.5-131.0)	112.5 (78.0-204.0)*	71.0 (54.5-116.0)
UAE (μg/mL)	72.3 ± 46.1*	32.4 ± 16.5	19.5 ± 6.1
ACR (μg/mg Cr)	50.6 ± 37.5*	20.5 ± 9.8	12.6 ± 4.3
eGFR (mL/min/1.73 m^2)	82.4 ± 19.3*	92.6 ± 20.1	93.2 ± 7.5
HsCRP (mg/dL)	0.11 (0.07-0.22)	0.45 (0.19-2.51)*	0.08 (0.06-0.25)

Data are expressed as mean ± SD, median (IQR), or frequency (%). *Significant (P < 0.001), compared with healthy individuals. NGAL: neutrophil gelatinase-associated lipocalin; UAE: urine albumin excretion; ACR: albumin-to-creatinine ratio; eGFR: estimated glomerular filtration rate; hsCRP: high-sensitivity C-reactive protein.

Table 5. Relationship between the plasma NGAL, NGAL/Inf ratio, renal function, and glycemic parameters of diabetic patients

Parameters	Multivariate linear regression analysis	
	Plasma NGAL	NGAL/Inf ratio
Renal function		
Serum creatinine (mg/dL)	0.426 (< 0.001)	0.581 (< 0.001)
eGFR (mL/min/1.73 m^2)	-0.382 (< 0.001)	-0.445 (< 0.001)
UAE (μg/mL)	0.245 (< 0.001)	0.437 (< 0.001)
Glycemic parameters		
HbA1c (%)	0.028 (0.729)	0.120 (0.205)
Fasting plasma glucose (mmol/L)	0.026 (0.809)	0.101 (0.341)

Data are expressed as standardized β (*P* value). Adjusted for age, gender, BMI, SBP, total cholesterol, and smoking habit. eGFR: estimated glomerular filtration rate; UAE: urine albumin excretion; HbA1c: glycated hemoglobin; NGAL: neutrophil gelatinase-associated lipocalin; NGAL/Inf ratio: NGAL-to-inflammation index ratio.

NGAL may not accurately reflect diabetic nephropathy.

NGAL is upregulated in renal tubular damage; thus, it is known as a tubular stress protein [20]. A study reported that tubular injury may precede microalbuminuria in diabetic patients [21]. Hence, NGAL appears to function as an early biomarker for diagnosing diabetic nephropathy. In a previous study, plasma NGAL independently reflected the degree of tubular damage in patients with diabetic kidney disease [22]. However, in the present study, there was no significant difference in plasma NGAL levels between patients with microalbuminuria and those with normoalbuminuria. Our results are consistent with the results of several studies showing that microalbuminuric subjects did not differ from normoalbuminuric subjects in terms of NGAL levels [23, 24]. These inconsistencies may reflect the differences in the severity of disease, presence of inflammation, onset of diabetes, and status of glycemic control among the studies.

Microalbuminuria is a sensitive indicator of glomerular injury and is regarded as a characteristic feature of the early stage of diabetic nephropathy [25, 26]. In the current study, to reduce the effect of inflammation on plasma NGAL, the NGAL/Inf ratio was calculated, and the use of the NGAL/Inf ratio for assessing microalbuminuria in T2DM was evaluated. The NGAL/Inf ratio of patients with microalbuminuria was significantly higher than that of patients with normoalbuminuria. Furthermore, in ROC curve analysis, the NGAL/Inf ratio demonstrated better diagnostic performance than plasma NGAL for identifying microalbuminuria. To evaluate the improvement of risk prediction by the NGAL/Inf ratio, the NRI and IDI were calculated. The NGAL/Inf ratio significantly improved risk prediction for diabetic nephropathy as measured by the NRI and IDI. These results suggest that the NGAL/Inf ratio may be superior to plasma NGAL in microalbuminuria prediction. A possible explanation for these findings is that falsely elevated NGAL levels could be corrected by adjustment with the inflammation index.

Screening for diabetic nephropathy should be initiated at the time of diagnosis for patients

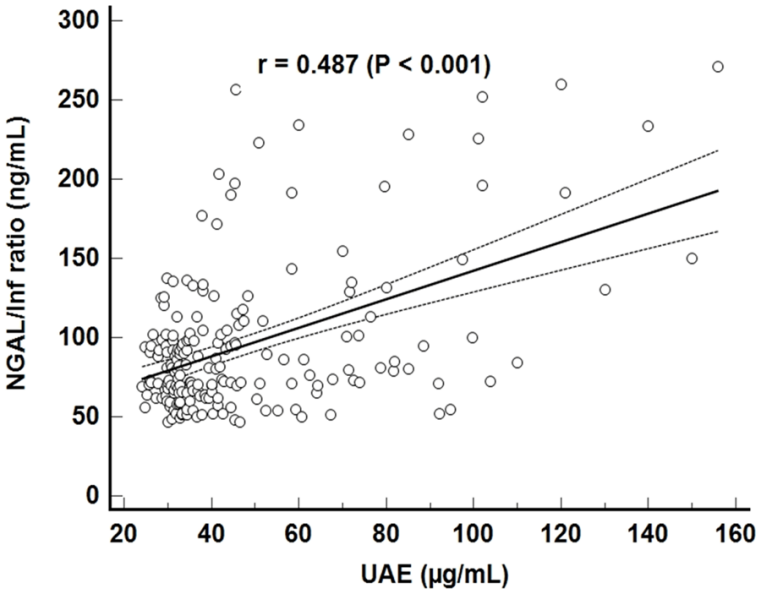

Figure 2. Scatter plots showing the relationship between the UAE and the NGAL/Inf ratio of diabetic patients. The NGAL/Inf ratio is significantly associated with UAE ($y = 0.902x + 52.164$, $r^2 = 0.237$, $P < 0.001$).

Table 6. Logistic regression analysis of risk factor for microalbuminuria in diabetic patients

Parameters	Adjusted odds ratio (95% CI)	*P* value
Diabetes duration	1.39 (1.06-2.29)	< 0.001
HbA1c	1.47 (1.08-2.62)	< 0.001
SBP	1.08 (1.00-1.75)	0.031
NGAL	1.05 (1.02-1.12)	0.037
NGAL/Inf ratio	1.16 (1.04-2.16)	0.025
NGAL/Inf ratio > 132 ng/mL	1.34 (1.09-2.54)	0.012

Adjusted for age, gender, BMI, total cholesterol, and smoking habit. HbA1c: glycated hemoglobin; SBP: systolic blood pressure; NGAL: neutrophil gelatinase-associated lipocalin; NGAL/Inf ratio: NGAL-to-inflammation index ratio; CI: confidence interval.

with T2DM because approximately 7% of them already have microalbuminuria at the time of diagnosis [27]. After being diagnosed with T2DM, around 2% of patients have been reported to experience progression to microalbuminuria annually [28]. Typical risk factors associated with the development of microalbuminuria include hyperglycemia, hypertension, dyslipidemia, and smoking habit [29]. This study evaluated the relationship between NGAL, the NGAL/Inf ratio, and UAE in T2DM. Serum creatinine and UAE levels were more significantly associated with the NGAL/Inf ratio than plasma NGAL. After adjusting for potential confounders, multivariate logistic regression analysis revealed that an elevated NGAL/Inf ratio > 132 ng/mL led to a 1.34-fold increase in the risk for microalbuminuria. Therefore, compared with plasma NGAL, an elevated NGAL/Inf ratio may be more closely associated with renal dysfunction and more accurately reflect the development of diabetic nephropathy in patients with T2DM.

NGAL is recognized as an acute phase protein because it is released by activated granulocytes under inflammatory conditions [30]. However, it is unclear whether NGAL plays a more important role as an indicator of inflammation than as a biomarker of kidney injury [31]. In this study, we examined whether renal dysfunction or inflammation contributes more to NGAL elevation in T2DM. When patients with renal dysfunction were excluded from the sample population, the level of plasma NGAL remained higher in diabetics than in healthy individuals. However, when patients with inflammation were excluded, the level of NGAL was similar to that in the control group. Based on the findings, inflammation may play a crucial role in enhanced NGAL production, at least in our patients.

This study further examined to what extent inflammation and renal dysfunction can affect the magnitude of NGAL elevation in diabetics. The percent difference between NGAL and the NGAL/Inf ratio of diabetic patients was 34.5%, which was significantly greater compared with that between NGAL and the NGAL/eGFR-index (13.4%). The results suggest that inflammation may contribute more than kidney function to NGAL elevation. Furthermore, the uncorrected NGAL level should be used with caution when assessing diabetic nephropathy in T2DM, par-

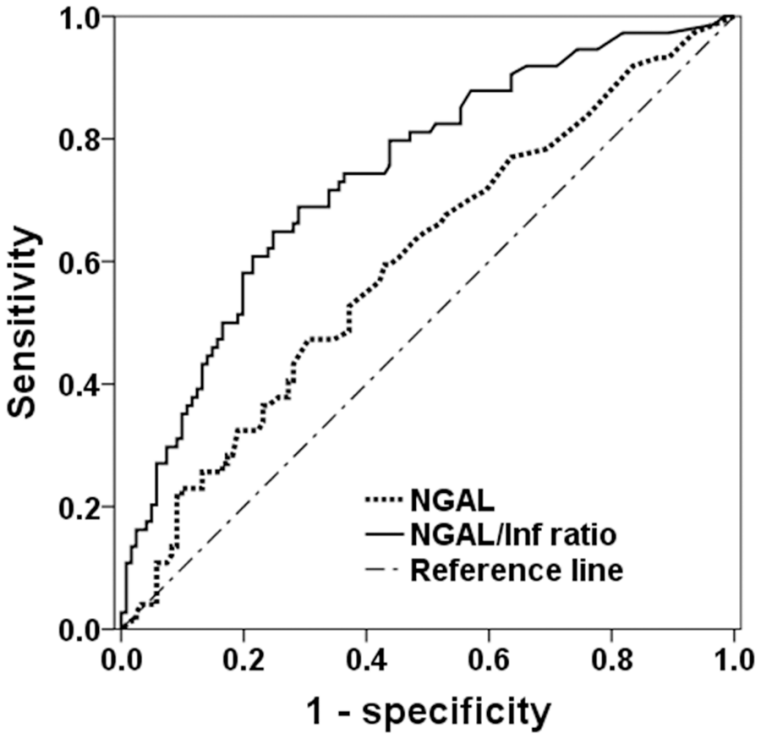

Figure 3. ROC curve analysis demonstrating the diagnostic efficacy of plasma NGAL and the NGAL/Inf ratio for identifying microalbuminuria in patients with T2DM. The AUC of the NGAL/Inf ratio (AUC, 0.742, 95% CI = 0.671-0.814; sensitivity 64.9%; specificity 75.2%; positive predictive value 61.5%; and negative predictive value 77.8% at the optimal cutoff of 132 ng/mL) is significantly larger than that of plasma NGAL (AUC, 0.599, 95% CI = 0.517-0.681; sensitivity 47.3%; specificity 69.4%; positive predictive value 48.6%; and negative predictive value 68.2% at the cutoff of 150 ng/mL; $P < 0.001$).

ticularly if patients have coexisting systemic inflammation. To the best of our knowledge, this is the first study to report the usefulness of the NGAL/Inf ratio for evaluating renal dysfunction in diabetic patients.

This study has several limitations. NGAL levels were not measured in serial samples to evaluate possible disease progression. The status of hypoxemia in diabetics, which might affect NGAL levels, was not assessed. As our investigation was a cross-sectional study, the evidence for a cause-and-effect relationship between NGAL and diabetic nephropathy was limited.

In conclusion, this study demonstrates that plasma NGAL levels in patients with T2DM may be overestimated due to systemic inflammation associated with diabetes. Therefore, plasma NGAL should be adjusted using the inflammation index for accurate renal dysfunction evaluation. In comparison with the direct use of plasma NGAL without correction, measurement of the NGAL/Inf ratio will be useful for assessing early diabetic nephropathy in patients with T2DM.

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Disclosure of conflict of interest

None.

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