Evaluation of serum Neutrophils Gelatinase–associated Lipocalin as a marker of diabetic nephropathy

By Kifah H. Jareh

WORD COUNT 6329
TIME SUBMITTED 14-JUN-2024 06:24PM
PAPER ID 109649249

Word count: 6,329

Evaluation of serum Neutrophils Gelatinase–associated Lipocalin as a marker of diabetic nephropathy

Kifah H. Jareh [1], Nazar Haddad [1], Safaa Gatea Mezban [2]

1 Department of Biochemistry, College of Medicine, University of Basrah, Basrah, Iraq
2 Department of Surgery, Urology and Renal Transplantation Center, Al-Sadder Teaching Hospital, Basrah Health Directorate, Ministry of Health, Basrah, Iraq

Corresponding author:
Mezban SG
E-mail: Medicalresearch55@yahoo.com

ABSTRACT

Background: One of the most prevalent and serious microvascular consequences of diabetes mellitus is diabetic nephropathy (DN). To optimize case outcomes, it is crucial to identify DN early and accurately. Recently, tubulo-interstitial indicators have gained attention as a potential new tool in the fight against diabetic kidney disease (DKD) in its early stages. One potential marker for renal function evaluation in diabetes mellitus cases is neutrophil gelatinase-associated lipocalin (NGAL).

Objective: This study aims to evaluate the diagnostic effectiveness of serum neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for early detection of nephropathy in persons with both type 1 and type 2 diabetes mellitus.

Methods: A case-control study was conducted at Faihaa teaching hospital (Faihaa specialist diabetes, endocrine, and metabolism center) between January 2019 and December 2019. A total of 160 diabetic cases were categorized into three groups based on their urine albumin to creatinine ratio (ACR) levels: 61 with normoalbuminuria, 69 with microalbuminuria, and 40 with macroalbuminuria. The study also included thirty-five control patients who did not have diabetes. Every participant had a comprehensive medical assessment. Neutrophil gelatinase associated lipocalin (NGAL), fasting serum glucose (FSG), urea, serum creatinine, serum albumin, and HBA1c levels were assessed. The urinary albumin to creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) were computed.

Results: Level of serum NGAL were significantly elevated diabetic cases as compared to controls as well as macro and micoalbuminuric cases had significant high levels as compared to

normoalbuminuric cases, out of 160 diabetic cases, 61 had normoalbuminuric, 69 had microalbuminuria, and 40 had macroalbuminuria, classified by their urine albumin to creatinine ratio (ACR) ratios. The study also included 35 control patients who did not have diabetes. The ROC analysis revealed that a serum NGAL threshold of 223.06 ng/mL exhibited a sensitivity of 90.9%, specificity of 78.3%, and an area under the curve (AUC) of 0.938. Moreover, when predicting the presence of nephropathy, an ACR of 30.24 mg/g exhibited a sensitivity of 98%, specificity of 100%, and an AUC of 0.999.

Conclusion: Diabetic patients, whether they have albuminuria or not, have substantially elevated serum neutrophil gelatinase related lipocalin, which suggests early tubular injury. Albuminuria is a strong predictor of this. NGAL testing has the potential to be a valuable technique for assessing diabetic renal involvement and detecting nephropathy at an early stage.

Keywords: diabetic nephropathy, serum neutrophil gelatinase associated lipocalin, albumin to creatinine ratio

INTRODUCTION

Diabetes mellitus (DM) is a prevalent global health concern that is projected to affect around 463 million adults worldwide. The estimated figure for this number is expected to rise to 578 million by 2030 and then increase to 700 million by 2045 [1]. In 2019, the International Diabetic Federation (IDF) determined that the Middle East and North Africa have the highest prevalence of diabetes among all IDF areas, with an estimated rate of roughly 12.2%. This percentage is expected to increase to 13.9% by 2045 in the Middle East and North Africa, and 9.6% worldwide. Specifically in Iraq, it is projected that 3.784 million individuals will die because of diabetes mellitus by 2045 [2]. DM defined as a group of common metabolic disorders that refer to hyperglycemia associated with other biochemical disturbances and the existence of gradual tissue damage with both micro-vascular and macro-vascular complications [3].

Diabetes is responsible for causing end-stage renal disease (ESRD) in approximately 45% of cases that require dialysis [4, 5]. Diabetic nephropathy (DN) or diabetic kidney disease is characterized by the increased excretion of albumin in urine, the formation of glomerular lesions due to diabetes, and a decrease in glomerular filtration rate (GFR). Diabetic nephropathy has emerged as the primary cause of end-stage renal disease (ESRD) in many countries because of the widespread prevalence of diabetes [6].

The annual occurrence rate of diabetic nephropathy is significantly elevated (3%) within the initial 10 to 20 years following the initiation of diabetes [7]. Approximately 20 to 40% of individuals with diabetes are expected to develop chronic kidney disease (CKD) [8], Given the size of the population,

a significant number of people are likely to develop end stage kidney disease (ESKD), which requires the use of renal replacement therapies such as dialysis or kidney transplantation [9].

Diabetic nephropathy can be categorized into various phases based on the presence of albuminuria and the level of renal dysfunction. Microalbuminuria is currently recognized as an initial phase of diabetic nephropathy, rather than a forecaster of it [10, 11].

Multiple epidemiological studies provide evidence that ethnicity, family history, hypertension, dyslipidemia, obesity, and insulin resistance are the primary risk factors for diabetic nephropathy [12]. Additional potential risk factors consist of increased levels of glycated hemoglobin (HbA1c), proteinuria, and smoking [13]. Until recently, the diagnosis of diabetic nephropathy required the presence of albuminuria of at least 300 mg/day in two out of three consecutive samples taken 3-6 months apart [14]. Urinary albumin is acknowledged as an early indicator of DN, however, substantial damage to the glomeruli has already taken place by the time albumin is detected in the urine.

Urinary albumin is acknowledged as an early indicator of DN, however, substantial damage to the glomeruli has already taken place by the time albumin is detected in the urine. Hence, there is a want for new urine biomarkers to detect individuals who are susceptible to kidney injury [15]. Neutrophil gelatinase-associated lipocalin (NGAL) is a biomarker that is used to detect renal tubular injury in the early stages. It is elevated in the distal tubules and collecting ducts of the kidneys. NGAL has been widely studied for its role in diagnosing acute kidney injury (AKI) [16].

A glycoprotein with 198 amino acids is called neutrophil gelatinase associated lipocalin (NGAL), human neutrophil lipocalin (HNL), alpha-1 microglobulin related protein, siderocalin, or uterocalin. It is sometimes called migration stimulating factor inhibitor (MSFI). The gene that makes it is located on the chromosome region. 3p11. The compound was initially extracted from mouse kidney cells that were infected with a simian virus known as SV-40 [17]. The NGAL gene consists of seven exons that generate a minimum of five functional transcripts, namely messenger RNAs (mRNAs) that are subsequently translated into proteins. Among these transcripts, some are responsible for encoding a secreted protein consisting of 198 amino acids [18].

The boxes reflect exons, which are the coding regions of a gene, while the connecting lines represent introns, which are the non-coding regions of a gene. Coding sequences are shown by filled-in boxes, whereas the untranslated region (UTR) is represented by unfilled boxes. The numerical value shown above the transcript represents the length of the completely formed transcript, indicated in terms of the number of base pairs. The number of amino acids is equal to the number of residues that undergo translation. The length of each transcript is directly correlated with the length of the genomic DNA [19].

Under normal physiological settings, one key role of NGAL is to function as a bacteriostatic agent [20]. NGAL is also known to serve as a chemoattractant for neutrophils and as a suppressor of cellular oxidative stress [21]. Furthermore, it has been observed to have an impact on the process of wound healing [22]. Additionally, it is involved in regulating the growth and production of cartilage by chondrocytes in mice, and it stimulates the proliferation of renal tubular epithelial cells [23]. In addition to affecting healthy cells, NGAL also influences the growth and survival of specific cancerous cells, such as those found in thyroid cancer and hepatocellular carcinoma [24].

In recent years, there has been a substantial surge in several research examining NGAL as a biomarker for both diagnosis and prognosis. NGAL has become a possible biomarker in various benign and malignant human diseases due to its secretive character and the presence of commercially available strong immunoassays [18].

Bolignano and his colleagues proposed the advantages of using it as a biomarker in DN and subsequently in chronic kidney disease (CKD) [25, 26]. NGAL levels are elevated in various conditions, including renal parenchyma disease, heart failure, endothelial dysfunction, preeclampsia, infection, inflammation, and malignancy. This rise in NGAL levels is observed irrespective of the glomerular filtration rate (GFR) [27-31].

NGAL levels were significantly higher in individuals with diabetes compared to non-diabetic control controls [32]. Moreover, there exists an inverse relationship between urinary NGAL and eGFR, while there is a direct relationship with serum Cystatin C (CysC), serum creatinine (SCr), and urinary albumin creatinine ratio (UACR). These findings indicate that urine NGAL has the potential to serve as a clinical indicator for the early detection of DN [33]. Additionally, there is a strong correlation between serum NGAL levels and the length of time a person has had diabetes, their ability to control blood sugar levels, and the presence of urinary interleukin-18 and angiotensinogen. This indicates that serum NGAL can be a valuable tool for assessing kidney damage in individuals with diabetes [34]. Individuals with diabetes, regardless of the presence of albuminuria, exhibit increased levels of serum NGAL. This suggests that there is early damage to the renal tubules and indicates that serum NGAL can be used as an early and sensitive indication of kidney dysfunction in diabetic cases without current evidence of nephropathy [35].

The objective of the study is to evaluate the diagnostic effectiveness of serum neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for early detection of nephropathy in persons with type 1 and 2 diabetes mellitus.

METHODS

Study Design

This study is a case-control study that included 160 consecutive cases with type 1 and type 2 diabetes mellitus (DM) who sought consultation at Faihaa Teaching Hospital-Faihaa Specialized Diabetes, Endocrine, and Metabolism Center from January 2019 to December 2019. Additionally, 45 healthy control subjects were randomly selected from the same center. The control subjects were enrolled in the study if they had a fasting serum glucose level of less than 100 mg/dl after an overnight fast and had no previous history of DM or renal diseases.

The group of individuals with diabetes were categorized into three groups based on their urine albumin/creatinine ratio (ACR). There were 61 cases with normoalbuminuric (ACR <30 mg/g creatinine), 59 cases with microalbuminuria (ACR ranging from 30 to 300 mg/g creatinine), and 40 cases with macroalbuminuria (ACR >300 mg/g creatinine).

The study received approval from the research ethical council of Basrah College of Medicine. All subjects provided verbal agreement before participating in the study.

Inclusion criteria

Any cases with diabetes mellitus regardless the duration of the disease for those case with type 2 DM, while those with type 1 must have duration more than 5 years. The control group consisted of normal healthy subjects with no history of diabetes mellitus or renal diseases, who agreed to participate and consented to this study.

Exclusion criteria

1) Renal failure
2) Pregnancy
3) Malignancy
4) Active urinary tract infection
5) History of dialysis or renal transplant
6)

Clinical evaluation

Each subject underwent full medical evaluation which include full medical history, focusing on the presence of comorbidities, and clinical examination.

All the cases' weight and height were measured. Body mass index (BMI) was calculated as the ratio of the weight to the height (kg/m2). Arterial blood pressure was measured three times following at least 10 min of rest with a mercury sphygmomanometer and the average value was considered for data analysis.

Glomerular filtration rate were estimated using 2009 Chronic Kidney Disease Epidemiology Collaboration formula based on serum creatinine (**CKD-EPICr**).

The first step in diagnosis and screening of diabetic nephropathy is to measure albumin in a spot urine sample. Urine sample is collected either as the first urine in the morning or at random. This method is easy to perform, accurate, and recommended by American Diabetes Association guidelines [14].

Analytical methods

Collection of blood samples

After 12 hours of overnight fasting, venous blood samples were collected from diabetic cases and controls into two types of vacutainer tubes and processed as follows: first tube with ethylene-diaminetetraacetic acid (lavender cap) without centrifugation (whole blood sample) for assaying glycosylated hemoglobin; and second tube gel tube (yellow cap) and allowed to clot for 2 hours at room temperature then centrifuged for 15 minute at 1000×g. Serum was rapidly separated and subdivided into aliquots. One aliquot of serum was used to measure glucose, urea, creatinine, albumin, and ALT on the same day as the blood was collected. The remaining aliquots of serum was transferred into plain tube labelled by name and date and stored at −20 °C for NGAL determinations. Hemolyzed samples were excluded.

***Estimation of serum* NGAL**

Serum NGAL levels were measured using a commercially available ELISA (Bioporto, Gentofte, Denmark). The intra- and inter-assay coefficients of variation (CVs) were 11% and 4%, respectively.

Estimation of albuminurea

Spot urine (clean-catch midstream urine) sample were obtained at morning, using disposable clean dry cups without preservatives. Every specimen was divided into 2portions. The first portion was for immediate urine examination which included dipsticks and microscopic examination .Urine Protein was measured by Dipsticks using Uro Coombi screen 11 sys plus reagent strips. Test results were used as a primary rough method for determination of albuminuria. The second portion was refrigerated at -20°C to be used for the accurate assay of urinary albumin on other day, by the fully-auto chemiluminescence immunoassay (CLIA) Analyzer integra 400.

ACR was estimated by dividing the albumin concentration in mg/L over the urine creatinine concentration in grams per liter. ACR was thus expressed in mg/g creatinine.

Other laboratory test

Fasting serum glucose (FSG) concentration was determined (by using hexokinase method), serum urea, creatinine, albumin, and ALT were measured using INTEGRA Germany 400 PLUS analyzer by Roche Diagnostics GmbH, Mannheim). HbA1c was determined using high-performance liquid chromatography (Variant™ II; Bio-Rad).

Statistical Analysis

Statistical calculations were done using Statistical Package for the Social Sciences version 25 (SPSS Inc.). In which categorical data expressed as numbers and percentages, and the differences between the groups were analyzed using Chi-square test (x2). Continuous data expressed as medians or mean ± SD and the differences between the groups were analyzed by one-way analysis of variance (ANOVA) and independent sample t test for normally distributed data and non-parametric Kruskal–Wallis H test and Mann-Whitney U test for not normally distributed data. Shapiro-Wilk test used to assess normality of the data, and outliers were detected using Boxplot methods, and Levene's test used to assess the homogeneity. In case of using ANOVA test, Post hoc testing was performed by the Games-Howelland and Tukey's-b tests to compare the difference among the studied groups. The correlations between various variables were calculated using the Pearson correlation coefficient. A receiver operating characteristic (ROC) analysis was employed to calculate the area under the curve (AUC) and find the best cut-off values to maximize diagnostic specificity and, secondarily, sensitivity. P-values <0.05 were accepted as statistically significant.

RESULTS

A total of 205 subjects were enrolled in the study, 45 non diabetic (controls) subjects and 160 diabetic cases. The mean age for diabetics were (49.18±15.2 year) and for non-diabetics were (45.44±21.6 year). Age, gender, and BMI shows no statistical significant (p value >0.05). While SBP, DBP, HbA1c, s. albumin, and FBG were significantly differs between diabetics and non-diabetics subjects (**Table 1)**

Table 1. Comparison between diabetics and non-diabetics regarding anthropometric and laboratory parameters.

Variables		**Diabetic cases**	**Non-Diabetic Cases**	**P value**
Age (years) (mean)		49.18±15.2	45.44±21.6	0.48
Gender	Male	74(46.3%)	15 (33.3%)	0.13

	Female	86 (53.8%)	30(66.7%)	
Duration(years)(median)		7.0	-	
BMI(kg/m2) (mean±SD)		28.9±5.88	28.2±7.58	0.524
SBP (mm/Hg) (median)		140.0	122	<0.001
DBP(mm/Hg) (mean±SD)		83.95±10.1	79.78±11.8	0.02
HbA1c (%)(mean±SD)		10.3±2.34	5.28±.54	<0.001
S. Albumin g/dl (median)		4.4	4.8	<0.001
FBG(mg/dl) (median)		192.5	82	<0.001

On the other hand, B. urea, creatinine, ACR, GFR, and NGAL showed highly significant differences between diabetics and non-diabetics (**Table 2**).

Table 2. Comparison between diabetics and non-diabetics regarding blood urea, creatinine, ACR, GFR, and S.NGAL

Variables	Diabetic cases	Non-Diabetic Cases	P value
B.urea(mg/dl)(median)	29	21	<0.001
S. creatinine(mg/dl) (median)	0.7	0.6	<0.001
ACR (mg/g) (median)	47.25	12.5	<0.001
GFR (mL/mim/1.73 m2 (median)	100.8	126.4	<0.001
SNGAL(ng/ml) (median)	300	65.25	<0.001

The groups that were analyzed did not show any statistically significant differences (P <0.05) in BMI and duration of diabetes. However, there was a significant rise in both systolic and diastolic blood pressure values in individuals with micro- and macroalbuminuria when compared to the control group. In addition, the instances with macroalbuminuria showed a notable difference in systolic blood pressure levels compared to the cases with normoalbuminuria. Moreover, there was a significant difference in diastolic blood pressure measurements between persons with micro- and macroalbuminuria compared to those with normoalbuminuria. There were significant differences in FBG (fasting blood glucose), blood urea, and HbA1c% levels between different groups of diabetics (normoalbuminuric, microalbuminuric, and macroalbuminuric cases) compared to the non-diabetic group (controls). Additionally, the macroalbuminuric cases showed a significant difference in blood urea levels compared to the normoalbuminuric cases **(Table 3).**

Regarding serum albumin, macroalbuminuric cases significantly differs from that of controls, normo- and microalbuminuric cases. While control group shows significant differences with micro- and normoalbuminuric cases in comparison with controls.

Macroalbuminuric cases shows a significant higher medians in term of serum creatinin and ACR, and lower medians of GFR compared to controls, micro- and normoalbuminuric cases. While microalbuminuric cases significantly differs from controls and normoalbuminuric in case of ACR.

On other hand GFR shows significantly higher levels among controls in comparison with micro- and normoalbuminuric cases.

Regarding s. NGAL, controls group was significantly differs from normo-, micro- and macroalbuminuric cases. While normoalbuminuric cases significantly differs from micro- and macroalbuminuric cases (**Table 4**).

Table 3. Statistical analysis of anthropometric and laboratory data from the various study groups.

Variables		Controls	Normoalbuminurea	Microalbuminurea	Macroalbuminurea
Age(years)(mean±SD)		45.4±21.6	47.08±16.8	49.8±12.1	51.3±16.4
Gender	Male	15 (33.3%) ‡	35 (57.4%) *,‡,†,‡	21 (35.6%) ‡	18 (45.0%) ‡
	Female	30 (66.7%) ‡	26 (42.6%) *,‡,†,‡	38 (64.4%) ‡	22 (55.0%) ‡
Duration(years)(median)		------	6.0	6.0	9.0
BMI (kg/m2)(mean±SD)		28.2±7.58	27.6±5.44	30.2±5.67	28.8±6.48
SBP(mm/hg)(median)		122†,‡	133‡	140*	156*,‡
DBP(mm/hg)(mean±SD)		79.7±11.8†,‡	80±9.3†,‡	85.5±9.5*,‡	87.5±10.3*,‡
HbA1c (%) (mean±SD)		5.2±.54‡,†,‡	10.2±2.29*	10.3±2.49*	10.4±2.21*
S. Albumin(g/dl) (median)		4.8‡,†	4.6*,‡	4.5*,‡	4.0*,‡,†
FBG(mg/dl)(median)		82‡,†,‡	179*	205*	200*

Table 4. Evaluation of blood urea, s. creatinine, ACR, GFR, and S. NGAL among the several groups that were part of the study

Variables	Controls	Normoalbuminurea	Microalbuminurea	Macroalbuminurea
B.urea(median) mg/dl	21‡,†,‡	28‡	28*	36*,‡
S. creatinine(median) mg/dl	0.6‡	0.7‡	0.7‡	1.02*,‡,†
ACR(median) mg/g	12.5†,‡	9.3†,‡	70.9*,‡,‡	952.67*,‡
GFR(median)	126.44‡,†	107.43*,‡	104.58*,‡	75.15*,‡,†

SNGAL(median) ng/ml	65.25[*,†,‡]	190[*,†,‡]	410.9[*,‡]	491.35[*,‡,]

ROC curves were utilized to evaluate the specificity and sensitivity of s. NGAL as a diagnostic tool for nephropathy detection, in comparison to ACR. Finding out whether s. NGAL is more specific or sensitive than ACR in this diagnostic context was the goal of the analysis **Table (5)** displays a juxtaposition of the output data from ROC curves for NGAL and ACR. A threshold of NGAL 223.06 ng/mL was established to differentiate between diabetic subjects with and without nephropathy. In the end, a cutoff of ACR 30.24 mg/g was determined to distinguish diabetics with nephropathy from those without. We can detect nephropathy with a sensitivity of 90.9% and a specificity of 78.3% when the blood NGAL level is 223.06 ng/mL. An AUC of 0.938 indicates good performance. Conversely, a sensitivity of 98% and specificity of 100% can be achieved with an albumin-to-creatinine ratio (ACR) of 30.24 mg/g in predicting the presence of nephritis. With an area under the curve of 0.999. Figure 1.

Table 5. Comparisons of NGAL and GFR ROC curve characteristics in nephropathy- and non-nephropathy-related patients.

Variables	Cut-off point	Sensitivity	Specificity	AUC	P value
SNGAL (ng/ml)	222.95	90.9%	77.3%	93.8	<0.001
	223.06	90.9%	78.3%		
	223.21	89.9%	78.3%		
	225.60	89.9%	79.2%		
	228.95	89.9%	80.1%		
ACR (mg/g)	29.05	99%	99.1%	99.9	<0.001
	29.97	99%	100%		
	30.24	98%	100%		
	30.84	97%	100%		
	31.81	96%	100%		
	32.39	94.9%	100%		

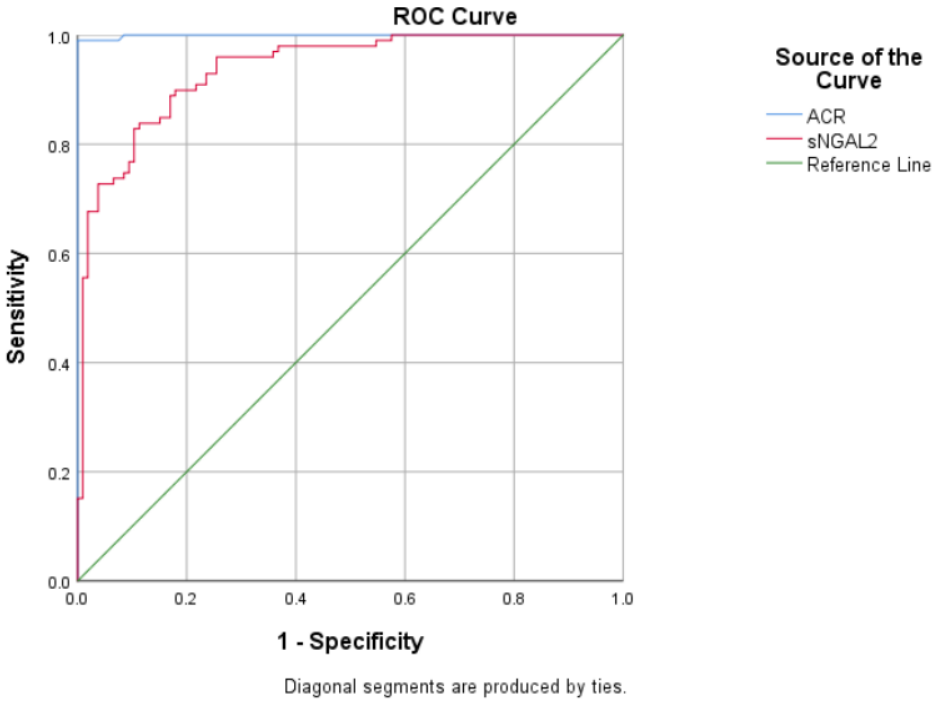

Figure 3.1 NGAL and GFR ROC curves for nephropathy and non-nephropathy cases, respectively.

The correlations between NGAL and GFR and ACR variables were examined in diabetic nephropathy cases using Pearson correlation analysis. The results are presented in Table 6. The Pearson correlation analysis revealed a significant association between NGAL concentration and GFR (r = -0.421, P = 0.0001) as well as ACR (r = 0.250, P = 0.0001). The glomerular filtration rate (GFR) exhibited a negative correlation with the albumin-to-creatinine ratio (ACR), with a correlation coefficient (r) of -0.459 and a p-value of 0.0001.

Table 6. Correlations of NGAL, GFR and ACR variables in studied groups

VARIABLES		GFR	ACR	SNGAL
NGAL	Correlation Coefficient	-0.421**	0.250**	1
	P value	<0.001	<0.001	--------
	No.	205	205	205
GFR	Correlation Coefficient	1	-0.459**	-0.421**
	P value	-	<0.001	<0.001

	No.	205	205	205
ACR	Correlation Coefficient	-0.459**	1	0.250**
	P value	<0.001	-	<0.001
	No.	205	205	205

Table 7 Showed the relationships between NGAL and other variables studied in diabetic nephropathy patients. There were strong positive associations for NGAL concentrations with age, FSG, Urea, Creatinine, HbA1c, SBP, and DBP, according to the Pearson correlation studies. However, it shows a negative relationship with serum albumin.

Table 7. The relationships between NGAL and other biochemical markers in diabetic nephropathy cases.

Variables	Correlation Coefficient	P value
NGAL-age	0313**	<0.001
NGAL-duration	-0.047	0.557
NGAL-FSG	0.256**	<0.001
NGAL-S. Albumin	-0.262**	<0.001
NGAL-ALT	-0.011	0.873
NGAL-B. Urea	0.414**	<0.001
NGAL- S. Creatinine	0.337**	<0.001
NGAL-BMI	0.088	0.212
NGAL-HbA1c	0.263**	<0.001
NGAL-SBP	0.29**	<0.001
NGAL-DBP	0.167*	0.017

At the cut-off value of NGAL 223 ng/mL, the number of TN (83) (90.2%), FP (23) (20.4%), TP (90) (79.6%), and FN (9) (9.8%). At the cut-off value of ACR 30 mg/g, the number of TN (106) (99.1%), FP (0) (0%), TP (98) (100%), and FN (1) (0.9%) (**Table 8**).

Table 8. TN, TP, FP, and FN for S.NGAL and ACR for cases with and without nephropathy

Variables	**Cut off point**	**Cases without nephropathy**		**Cases with nephropathy**	
		TN*	**FP***	**TP***	**FN***
s.NGAL	**223**	(83) 90.2%	(23) 20.4%	(90) 79.6%	(9) 9.8%

ACR	**30**	(106) 99.1%	(0) 0%	(98) 100%	(1) 0.9%

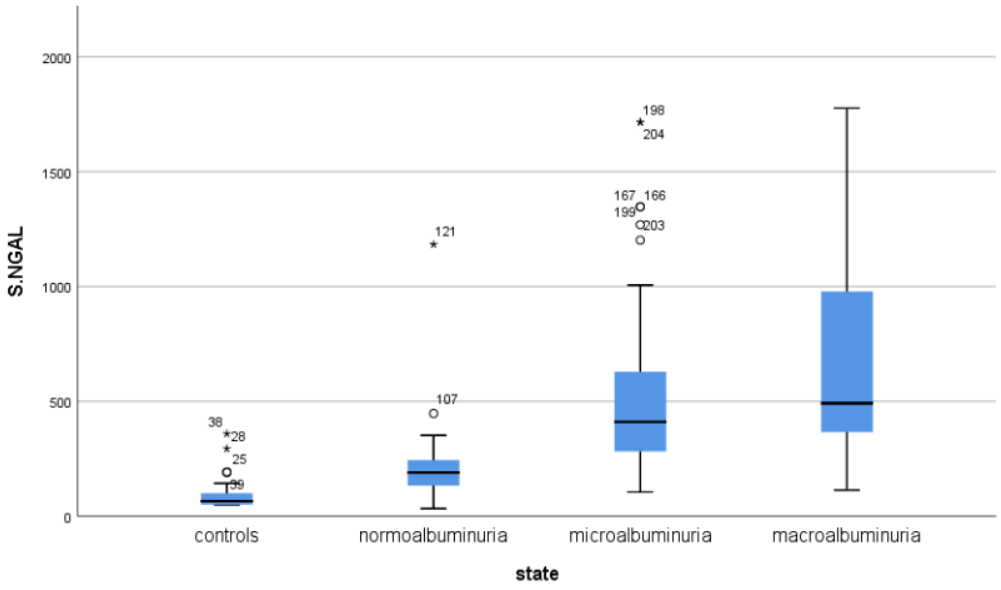

Figure 2. Box plot of S.NGAL values distribution among diabetic albuminurea state.

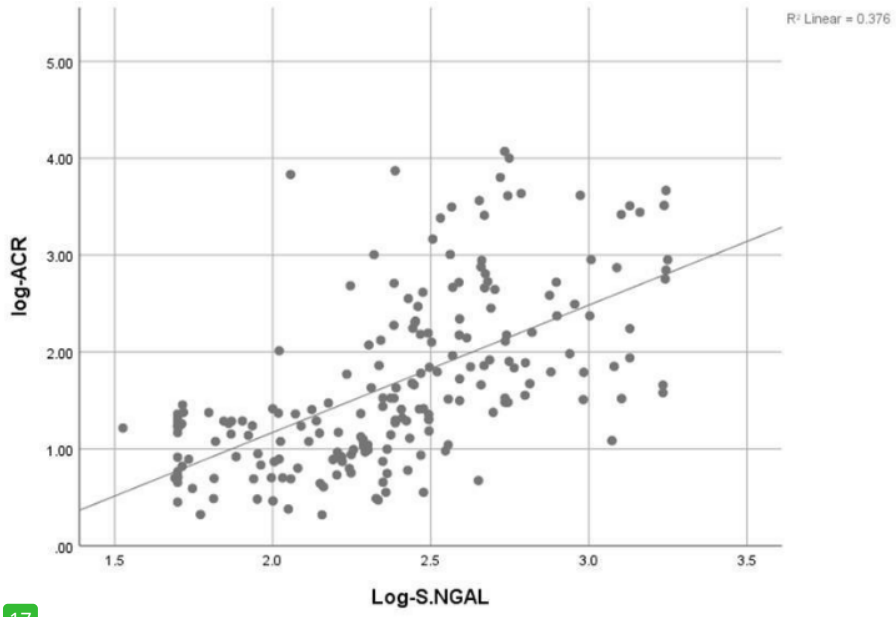

Figure 3. Scatter dots showing the correlation between Log-ACR and Log-S.NGAL

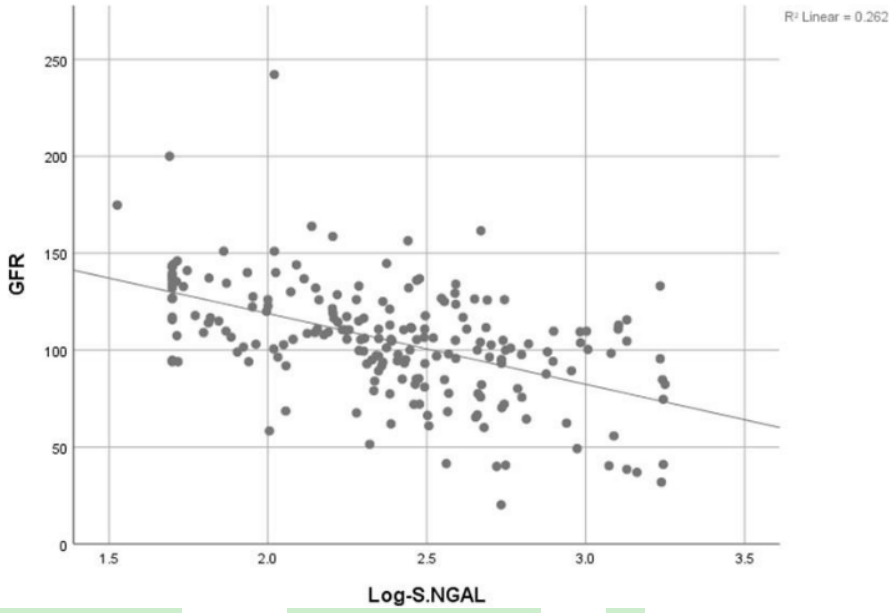

Figure 3. Scatter dots showing the correlation between GFR and Log-S.NGAL.

Discussion

One of the leading causes of chronic kidney disease and end-stage renal failure worldwide is diabetic nephropathy. Diabetic glomerular lesions, a reduction in glomerular filtration rate (GFR), and aberrant accumulations of urine albumin excretion are the hallmarks of this illness [36]. There was no discernible disparity in the ages of the groups under investigation.

The levels of HbA1c and FSG were significantly higher in the diabetes groups compared to the control group in this study. Sun et al. previously found that hyperglycemia is the main cause of diabetic nephropathy, and their results are in line with that conclusion [37]. In addition, Kundu et al conducted a study that showed significantly elevated levels of HbA1c in individuals with diabetes compared to those without diabetes. These elevations were found to be highly statistically significant ($P < 0.001$). Furthermore, the study revealed that such high levels of HbA1c related to the development of microangiopathy in individuals with diabetes. Kundu et colleagues further shown that individuals with diabetes who have inadequate metabolic control are at a higher risk of developing renal impairment, resulting in increased levels of microalbumin [38]. Zakkerkish and his colleagues [39] proposed that inadequate management of blood sugar levels may have a key influence in the advancement of diabetic nephropathy in individuals with type 2 diabetes mellitus (T2DM).

The results indicated that the control group had higher levels of GFR compared to both the micro-albuminuria and normoalbuminuric diabetes groups. The levels of ACR were significantly higher in the macroalbuminuric diabetes group compared to the control group, as well as the micro-albuminuria and normoalbuminuric diabetic groups.

According to Abid et al. [40], microalbuminuria can be diagnosed in type 2 diabetes mellitus (T2DM) patients when there are increased levels of urine albumin and albumin-to-creatinine ratio (ACR), as well as decreased glomerular filtration rate (GFR). The results show that compared to the control and normoalbuminuric diabetic groups, the microalbuminuric and macroalbuminuric diabetic groups had lower GFR values. The ACR values were also greater in the diabetes groups with microalbuminuria and macroalbuminuria than in the control and normoalbuminuric diabetic groups. A 25-kDa molecule known as NGAL is delivered to the kidney tubules a few hours following contact with a dangerous and unfamiliar stimulant. A correlation between the severity of chronic kidney damage, as shown in glomerulonephritis and autosomal dominant polycystic kidney disease, and the tubular secretion of NGAL has been found [41]. In this study, micro-, normo-, and macroalbuminuric diabetic groups had significantly higher serum NGAL levels compared to the control group, and micro- and macroalbuminuric diabetic groups had higher ACR levels, showing that tubular damage occurs before albumin excretion in nephropathy cases. When the damage developed into nephropathy, NGAL also rose. Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels were found to be higher in the blood serum and urine of 56 patients diagnosed with Type 2 Diabetes Mellitus (T2DM) in a cohort study.

The degree of kidney injury was shown to be correlated with these elevated NGAL levels.[42]. A non-invasive method for assessing diabetic kidney damage has been developed, and one of its biomarkers, NGAL, is detectable in both serum and urine. Even before albumin shows up in the urine, it can aid in the early identification of diabetic nephropathy [43]. Even in the absence of albuminuria, a subsequent study by Nielsen et al. found that high concentrations of urine neutrophil gelatinase-associated lipocalin (uNGAL) in T1DM patients indicate the beginning of tubular injury [44]. Also, in diabetic patients, NGAL was highly correlated with GFR. Albuminuria is positively correlated with serum NGAL levels, according to our findings.

According to our findings, serum NGAL has a negative correlation with GFR and a positive correlation with albuminuria. The findings presented by Yang et al. [45] are consistent with this. Results showed that diabetics with normoalbuminuria had significantly higher serum NGAL levels than the control group. This provides more evidence that serum NGAL may be used to detect tubular injury prior to the development of microalbuminuria, a characteristic of glomerular injury. When comparing diabetics to a control group, Bolignano et al. found that sNGAL and uNGAL levels were much greater in the former. In addition, they found that diabetes patients had higher NGAL levels even when there were no early indicators of glomerular impairment [46]. From complete absence to a severe state, NGAL fluctuates with the progression of albuminuria. When comparing diabetics with normoalbuminuria to a non-diabetic control group, Nauta et al. found that NGAL levels were 1.5 times higher in the former. [47]. An early biomarker for normoalbuminuric diabetic patients, NGAL levels grow in persons with T1DM even before microalbuminuria develops, according to Lacquaniti et al [48]. To assess the early stages of kidney impairment caused by diabetes, measuring NGAL could be helpful. Children with diabetes who did not produce albuminuria had higher levels of NGAL in their serum and urine, according to research by Zachwieja et al. Furthermore, nephropathy can still be present even when albumin levels are normal in the urine; this condition is characterized by elevated concentrations of NGAL in the blood and urine [49]. One possible advantage of NGAL testing over microalbuminuria is its ability to assess kidney damage in diabetic youngsters more quickly. According to our findings, serum NGAL had a strong negative correlation with GFR but a notable favorable relationship with HbA1c and ACR. Our results agree with those of Fu et al., who found that serum NGAL was positively associated with albuminuria and significantly negatively related to GFR [50]. Furthermore, Woo et al. discovered a negative correlation between serum NGAL and GFR [51].

Using receiver operating characteristic (ROC) curves, the diagnostic performance of NGAL was evaluated, and it was found that NGAL demonstrates exceptional sensitivity and specificity in the early diagnosis of DN. To determining NGAL's diagnostic effectiveness in DN, Chen and colleagues performed a meta-analysis. The study suggests that NGAL is a reliable biomarker for nephropathy

cases and that its diagnostic accuracy is higher for individuals with both microalbuminuria and macroalbuminuria compared to those with only microalbuminuria. With an area under the curve (AUC) of 0.92 and a 95% confidence interval (CI) of 0.90-0.94, NGAL was also shown to have a substantial diagnostic value for cases of type 2 diabetes mellitus which included microalbuminuria and macroalbuminuria [52].

CONCLUSION

The severity of renal impairment induced by diabetic disease is adequately reflected by the elevated sNGAL levels observed in diabetic individuals. This is why NGAL can be the first biomarker to identify high-risk diabetic nephropathy. One of the most important ways to detect diabetic nephropathy early on is using serum NGAL.

Disclosure

None

REFERENCES

1. IDF Diabetes Atlas (9th ed.), International Diabetes Federation, Brussels, Belgium (2019).
2. Wild S, Roglic G, Green a, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27:1047–1053.
3. American Diabetes Association, "10. Microvascular complications and foot care: standards of medical care in diabetes—2018," Diabetes Care. 2017; 41(1):105–118.
4. Stanton RC. Clinical challenges in diagnosis and management of diabetic kidney disease. Am J Kidney Dis. 2014; 63(2 Suppl 2):3–21.
5. O'Hare AM, Bertenthal D, Covinsky KE, Landefeld CS, Sen S, et al. Mortality risk stratification in chronic kidney disease: One size for all ages? J Am Soc Nephrol. 2006; 17:846–853.
6. Lim AKh. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis. 2014; 7:361–381.
7. Magee C, Grieve DJ, Watson CJ, Brazil DP. Diabetic nephropathy: a tangled web to unweave. Cardiovasc Drugs Ther. 2017;31(5–6):579–592
8. Papadopoulou-Marketou N, Paschou SA, Marketos N, Adamidi S, Ada-midis S, et al.Diabetic nephropathy in type 1 diabetes. Minerva Med. 2018; 109(3):218–228.
9. Nelson RG, Bennett PH, Beck GJ, Tan M, Knowler WC, et al. Diabetic Renal Disease Study Group: development and progression of renal disease in Pima Indians with non-insulin dependent diabetes mellitus. N Engl J Med. 1996; 335:1636–1642.
10. Can Z, Cooper ME. Pathogenesis of diabetic nephropathy. Journal of Diabetes Investigation. 2011; 2(4):243-247.
11. Schena FP, Gesualdo L. Pathogenetic mechanisms of diabetic nephropathy. J Am Soc Nephrol. 2005; 16:30–33.
12. Raile K, Galler A, Hofer S, Herbst A, Dunstheimer D, et al. Diabetic nephropathy in 27,805 children, adolescents, and adults with type 1 diabetes: effect of diabetes duration, A1C, hypertension, dyslipidemia, diabetes onset and sex. Diabetes Care. 2007; 30:2523–2528.
13. Eberhard R. Diabetic nephropathy. Saudi J Kidney Dis Transplant. 2006; 17:481–490.
14. American Diabetes Association. Nephropathy in diabetes (Position State- ment). Diabetes Care. 2004;27(Suppl. 1):79–83
15. Sulaiman MK. Diabetic nephropathy: recent advances in pathophysiology and challenges in dietary management. Diabetol Metab Syndr.2019;11:7.
16. Bonventre JV. Diagnosis of acute kidney injury: From classic parameters to new biomarkers. Contrib Nephrol. 2007; 156:213–219.

17. Hraba-Renevey S, Turler H, Kress M, Salomon C, Weil R. SV40-induced expression of mouse gene 243 involves a post-transcriptional mechanism. Oncogene. 1989; 4:601-608.
18. Chakraborty S, Kaur S, Guha S, Batra SK. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. Biochim Biophys Acta. 2012; 1826(1):129–169.
19. Brown TA. Genomes. 2nd edition. Oxford: Wiley-Liss; 2002. Chapter 7, Understanding a Genome Sequence.
20. Nairz M, Theurl I, Schroll A, Theurl M, Fritsche G, et al. Absence of functional Hfe protects mice from invasive Salmonella enterica serovar Typhimurium infection via induction of lipocalin-2. Blood. 2009; 114:3642-3651.
21. Bahmani P, Halabian R, Rouhbakhsh M, Roushandeh AM, Masroori N, et al. Neutrophil gelatinase-associated lipocalin induces the expression of heme oxygenase-1 and superoxide dismutase 1, 2. Cell Stress Chaperones. 2010; 15:395-403.
22. Pukstad BS, Ryan L, Flo TH, Stenvik J, Moseley R, et al. Non-healing is associated with persistent stimulation of the innate immune response in chronic venous leg ulcers. J Dermatol Sci. 2010; 59:115-122.
23. Owen HC, Roberts SJ, Ahmed SF, Farquharson C. Dexamethasone-induced expression of the glucocorticoid response gene lipocalin 2 in chondrocytes. Am J Physiol Endocrinol Metab. 2008; 294:1023-1034.
24. Lee EK, Kim HJ, Lee KJ, Lee HJ, Lee JS, et al. Inhibition of the proliferation and invasion of hepatocellular carcinoma cells by lipocalin 2 through blockade of JNK and PI3K/Akt signaling. Int J Oncol. 2011; 38:325-333.
25. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, et al. Neutrophil gelatinase- associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol. 2009; 4:337–344.
26. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Fazio MR, et al. Neutrophil gelatinase- associated lipocalin as an early biomarker of nephropathy in diabetic cases. Kidney Blood Press Res. 2009; 32:91–98.
27. Damman K, Van Veldhuisen DJ, Navis G, Vaidya VS, Smilde TD, et al. Tubular damage in chronic systolic heart failure is associated with reduced survival independent of glomerular filtration rate. Heart. 2010; 96:1297–1302.
28. D'Anna R, Santamaria A, Interdonato ML, Licata G, Giordano D. NGAL, an early marker of endothelial dysfunction in preeclampsia. Pregnancy Hypertens. 2011; 1:243.

29. Kim SM, Park JS, Norwitz ER, Jung HJ, Kim BJ, et al. Circulating levels of neutrophil gelatinase-associated lipocalin (NGAL) correlate with the presence and severity of preeclampsia. Reprod Sci. 2013; 20:1083–1089.
30. Petla LT, Chikkala R, Ratnakar KS, Kodati V, Sritharan V. Biomarkers for the management of pre-eclampsia in pregnant women. Indian J Med Res. 2013; 138:60–67.
31. Nielsen BS, Borregaard N, Bundgaard JR, Timshel S, Sehested M, et al. Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases. Gut. 1996; 38:414–420.
32. Hafez MH, El-Mougy FAF, Makar SH, Abd El Shaheed SS. Detection of an earlier tubulopathy in diabetic nephropathy among children with normoalbuminuria. Iranian Journal of Kidney Diseases. 2015; 9(2):126–131.
33. Assal HS, Tawfeek S, Rasheed EA, El-Lebedy D, Thabet EH. Serum cystatin C and tubular urinary enzymes as biomarkers of renal dysfunction in type 2 diabetes mellitus," Clinical Medicine Insights: Endocrinology and Diabetes. 2013; 6:7–13.
34. Al-Refai AA, Tayel SI, Ragheb A, Dala AG, Zahran A. Urinary neutrophil gelatinase associated lipocalin as a marker of tubular damage in type 2 diabetic cases with and without albuminuria. Open Journal of Nephrology. 2014; 4(1):37– 46.
35. Demir K, Abaci A, ¨ Ume TK, Altincik A, Atli GC, ¨ ober EB. Evaluation of neutrophil gelatinase-associated lipocalin in normoalbuminuric normotensive type 1 diabetic adolescents. Journal of Pediatric Endocrinology and Metabolism. 2012; 25(5-6):517–523.
36. Lim AKh. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis. 2014; 7:361-381. Published 2014 Oct 15.
37. Wan TT, Li XF, Sun YM, Li YB, Su Y. Recent advances in understanding the biochemical and molecular mechanism of diabetic retinopathy. Biomed Pharmacother. 2015; 74:145-147.
38. Kundu D, Roy A, Mandal T, Bandyopadhyay U, Ghosh E, et al. Relation of microalbuminuria to glycosylated hemoglobin and duration of type 2 diabetes. Niger J Clin Pract. 2013; 16(2):216-220.
39. Zakkerkish M, Shahbazian HB, Shahbazian H, Latifi SM, Moravej Aleali A. Albuminuria and its correlates in type 2 diabetic cases. Iran J Kidney Dis. 2013; 7(4):268-276.
40. Abid O, Sun Q, Sugimoto K, Mercan D, Vincent JL. Predictive value of microalbuminuria in medical ICU cases: results of a pilot study. Chest. 2001; 120(6):1984-1988.
41. Papadopoulou-Marketou N, Skevaki C, Kosteria I, Peppa M, Chrousos GP, et al. NGAL and cystatin C: two possible early markers of diabetic nephropathy in young cases with type 1 diabetes mellitus: one year follow up. Hormones (Athens). 2015; 14(2):232-240.

42. Jiao LY, Guo Q, Song Z, Lucy G. Diagnostic value of urinary neutrophil gelatinase associated lipocalin and serum cystatin C on diabetic Nephropathy, Journal of Xinxiang Medical College. 2011; 5: 21.
43. Basturk T, Altuntaş Y, Kurklu A, Aydin L, Eren N, et al. Urinary N-acetyl B glucosaminidase as an earlier marker of diabetic nephropathy and influence of low-dose perindopril/indapamide combination. Ren Fail. 2006; 28(2):125-128.
44. Nielsen S E, Schjoedt K J, Astrup A S, Tarnow L, Lajer M, et al. Neutrophil Gelatinase- Associated Lipocalin (NGAL) and Kidney Injury Molecule 1 (KIM1) in cases with diabetic nephropathy: a cross-sectional study and the effects of lisinopril. Diabet Med. 2010; 27(10):1144-1150.
45. Yang Y H, He X J, Chen S R, Wang L, Li E M, et al. Changes of serum and urine neutrophil gelatinase-associated lipocalin in type-2 diabetic cases with nephropathy: one year observational follow-up study. Endocrine. 2009; 36(1):45-51.
46. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, et al. Neutrophil gelatinase- associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol. 2009; 4(2):337-344.
47. Nauta F L, Boertien W E, Bakker S J, van Goor H, van Oeveren W, et al. Glomerular and tubular damage markers are elevated in cases with diabetes. Diabetes Care. 2011; 34(4):975- 981.
48. Lacquaniti A, Donato V, Pintaudi B, Di Vieste G, Chirico V, et al. "Normoalbuminuric" diabetic nephropathy: tubular damage and NGAL. Acta Diabetol. 2013; 50(6):935-942.
49. Zachwieja J, Soltysiak J, Fichna P, Lipkowska K, Stankiewicz W, et al. Normal-range albuminuria does not exclude nephropathy in diabetic children [published correction appears in Pediatr Nephrol. 2010 Aug; 25(8):1581]. Pediatr Nephrol. 2010; 25(8):1445-1451.
50. Fu W J, Xiong S L, Fang Y G, Wen S, Chen M L, et al. Urinary tubular biomarkers in short- term type 2 diabetes mellitus cases: a cross-sectional study. Endocrine. 2012; 41(1):82-88.
51. Woo KS, Choi JL, Kim BR, Kim JE, An WS, et al. Urinary neutrophil gelatinase-associated lipocalin levels in comparison with glomerular filtration rate for evaluation of renal function in cases with diabetic chronic kidney disease. Diabetes Metab J. 2012; 36(4):307-313.
52. Chen B, Li Y, Liu Y, Zang C, Wu M, et al. Diagnostic value of neutrophil gelatinase-associated lipocalin in diabetic nephropathy: a meta-analysis. Ren Fail. 2019; 41(1):489-496.