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Assessment of Serum and Urine Neurophil Gelatinase-Associated Lipocalin (s-NGAL and u-NGAL) Level as a Predictive Factor of Disease Progression in Diabetic Nephropathy in Type 2 DM

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Introduction. Diabetic nephropathy (DN) is a major complication of diabetes Mellitus. Early detection and intervention of DN can slow its progression and improve patients' outcomes. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of tubular damage might become a useful biomarker for the evaluation of renal involvement in diabetic patients. We aimed to evaluate the serum and urine NGAL(s-NGAL and u-NGAL) in type 2 diabetic patients and its correlation with different stages of diabetic nephropathy.
Methods. This cross-sectional study was designed on 198 subjects consisted of 50 controls and 148 type 2 diabetes patients (50 normoalbuminuric, 58 microalbuminuric, and 40 macroalbuminuric). The study was conducted with measuring s-NGAL and u-NGAL, albumin and spot urine creatinine were also measured.
Results. A highly increased level of s-NGAL was detected in macroalbuminuric group compared with controls, normoalbuminurics and microalbuminurics ($P < .01$). Highly raised u-NGAL levels were observed in macroalbuminurics in comparison with controls ($P < .01$). ROC curve demonstrated the best sensitivity and specificity of s-NGAL/u-NGAL for the macroalbuminuric state (sensitivity, 26% and 60%; specificity, 98% and 72%; respectively), in which the best cut-off points for the detection of macroalbuminuric state for s-NGAL/u-NGAL were 300 ng/mL and 71.4 ng/mL, respectively.
Conclusion. Serum and urine-NGAL are elevated in type 2 diabetic patients, with or without albuminuria, s-NGAL level clearly correlates with severity of renal damage caused by DN and u-NGAL increases in macroalbuminuric state. S-NGAL could be a useful, noninvasive, available and practical test for evaluation of diabetic renal involvement. We could suggest u-NGAL as a probable predictor of macroalbuminuria.

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INTRODUCTION

Diabetes mellitus (DM) is one of the most problematic health issues[1] and as a common systemic disease leads to major chronic micro and macrovascular complications including peripheral neuropathy, nephropathy, retinopathy, and cardiovascular complications.[2] Diabetic nephropathy occurs in 30-40 of diabetic patients.[3] It is known by

albuminuria, hypertension, cardiovascular disease and a progressive decline in kidney function leading to End Stage Renal Disease (ESRD) over time.[3-5] Development of diabetic nephropathy could be prevented by early screening for albuminuria and other protective interventions.[3]

The presence of significant albuminuria designates the most common sign of early renal involvement, which can be detected clinically in diabetic subjects.[6] Two important causes for albuminuria are mainly glomerular damage and defective tubular reabsorption of albumin.[7] The development of renal dysfunction and the adverse outcomes are more associated with the renal tubulointerstitial injury than the severe degree of glomerular damages.[8]

On the other side, serum creatinine, which has been largely used to reflect the impaired renal function in diabetic nephropathy for a long time, is not sensitive enough to identify early alteration in renal function.[4] Therefore, a more specific indicator of tubular dysfunction is needed to detect diabetic nephropathy at an early stage, even before the appearance of microalbuminuria. Meanwhile urinary biomarkers are increased in diabetic patients in comparison with normal subjects, which could be a valuable indicator of early, specific and precise prediction of diabetic nephropathy.[9]

Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein of the lipocalin proteins, generated in epithelial cells and neutrophils of most tissues, and is specifically demonstrated as a sensitive biomarker of renal tubulointerstitial damage and acute or chronic kidney diseases.[10,11] The increase in serum or urine NGAL (s-NGAL, u-NGAL) levels usually precedes the increase in serum creatinine and could be considered as important emerging marker to predict kidney disease and its severity.[12,13]

It is quite clear that early detection of tubular damage by this new marker could help us to identify the high-risk population and could prevent or delay diabetic nephropathy. In the literature review, there was a lack of similar studies in our country. Therefore, this study was designed to evaluate the level of serum and urine NGAL in type II diabetic patients at different stages of diabetic nephropathy. Furthermore, we aimed to assess the correlation of these markers with state of albuminuria (the gold standard marker of glomerular damage) for early prediction of diabetic nephropathy.

MATERIALS AND METHODS

Patient and Control Subjects

This cross-sectional survey was designed on 212 subjects recruited from an outpatient clinic at the Institute of endocrinology and metabolism (Tehran, Iran) from 2017 to 2019. The subjects were enrolled through a convenience-sampling scheme, in which the diabetic patients were classified into the normoalbuminuria, microalbuminuria, and macroalbuminuria according to their state of albuminuria. Moreover, a control group of non-diabetic healthy individuals was selected. Twelve cases were missed due to low- study compliance.

The population consisted of 50 individuals in the control group and 148 individuals with type 2 diabetes; (50 subjects were normoalbuminuric (without albuminuria), 58 diabetics were microalbuminuric, and 40 diabetics had macroalbuminuria). The sample size was calculated by G power software (version 3.1), power = 90%, α = 5%, missing rate = 20%, and the s-NGAL and u-NGAL mean ± SD (95% CI) were used from Nielsen *et al.*'s study.[14]

All of the participants signed the written informed consent, after an explanation about the study design and procedures. The local ethics committee of Iran University of Medical Sciences approved the study protocol (IR.IUMS.FMD.REC.1397.092) (IR.IUMS.REC 1395.95-02-122-29026). Exclusion criteria included the use of medications (angiotensin receptor blockers (ARBs), angiotensin II converting enzyme inhibitors (ACEI), diltiazem, supplements and vitamins), pregnancy, any other systemic disease or malignancy and serum creatinine (Cr) level ≥ 1.5 mg/dL or Estimated Glomerular Filtration Rate (e-GFR) < 60 mL/min. All patients and controls had negative urine culture.

Demographic and clinical data of the patients were recorded including sex, age, history of the disease and medication use, history of documented diabetic retinopathy and hypertension, duration of diabetes, BMI, blood pressure, and the type of treatment for diabetes control.

The laboratory tests including fasting glucose, lipids, creatinine and glycosylated hemoglobin (HbA1C) were extracted from electronic files. Diabetes Mellitus (DM) was described as having two FBS ≥ 126 mg/dL according to WHO criteria for diagnosis of DM.[15]

Albuminuria was defined according to albumin to creatinine ratio (ACR) in random urine or 24 hours urine collection for albumin and creatinine. ACR greater than 30 mg/g or daily urine albumin greater than 30 mg/24h was defined as microalbuminuria and a ratio greater than 300 mg/g or daily urine albumin greater than 30 mg/24h was considered as macroalbuminuria, Estimated GFR (e-GFR) was calculated using the CKD-EPI formula.[16]

Patients were selected from those subjects, who had not been treated with any of the above-mentioned medications and were referred to clinic for the evaluation of proteinuria.

ELISA NGAL Assay

For u-NGAL level measurement; approximately 20 ml of urine, collected and stored at -70 ˚c until further measurement. U-NGAL level was measured using the "Human NGAL ELISA kit", (BIOPORTO diagnostics, Denmark). For the evaluation of s-NGAL level, 5 milliliters of venous blood was drawn and its serum was isolated. The isolated serum was stored in -80 °C after centrifusion. After collection of all samples the NGAL levels were measured using "Human NGAL ELISA kit", (BIOPORTO diagnostics, Denmark).

Statistical Analysis

Statistical analysis was performed using STATA (version 11, Chicago IL). Preliminary assessments revealed that u-NGAL, age, and duration of diabetes did not follow a normal distribution, so we used non-parametric tests for analyzing them. Median (interquartile range (IQR)) and frequency (%)) were used for describing continuous and categorical variables, and in the same order, p-values are drawn from the median, Mann-Whitney U test and chi-squared tests. Chi square test and linear regression were also used in the analyses. To evaluate the predictive ability of the categorizes according to the Alb/Cr ratio, we considered the control group as a reference test and the other three groups as index tests (normo/ micro/ macro) and the u-NGAL[17] was the predictor. $P < .05$ was considered statistically meaningful.

RESULTS

In the study period, 198 subjects were evaluated between 2017 and 2019. Totally 97 (49%) were female and mean (SD) for age was 51.6 (13.04). For the daily collected urine samples, the distribution of albumin [median (interquartile (IQ)) mg/24h] was 3.4 (2.5 to 9), 15(8.1 to 21), 105 (61 to 149), and 396 (357 to 630) ($P < .001$), for control, normoalbuminuria, microalbuminuria, and macroalbuminuria groups; respectively. All subjects had GFR more than 60 mL/min.

All the recruited subjects with complete data of u-NGAL and s-NGAL (index tests) as well as albuminuria (reference standard) were evaluated.

The diabetic patients were well matched in terms of gender and duration of diabetes, though; there were differences in terms of age, e-GFR, Alb/Cr ratio or daily urine albumin and serum creatinine. Demographic, baseline and laboratory data of the subjects are concisely shown in Table 1.

In this study the u-NGAL and s-NGAL levels were compared separately in normoalbuminuric,

Table 1. Demographic, Baseline and Laboratory Characteristics of the Control and Diabetic Groups

	Control Group (n = 50)	Normoalbuminuric (n = 50)	Microalbuminuric (n = 58)	Macroalbuminuric (n = 40)	*P* Value (for Trend)
Female, n (%)	26 (52)	29 (58)	26 (44.8)	16 (40)	> .05
Age, y	39.6 ± 15	55.8 ± 9.3	54.8 ± 9.5	56.6 ± 9.3	> .05
Diabetes Duration, y	–	9.2 ± 6.4	9.0 ± 5.1	10.0 ± 5.0	> .05
FBS, mg/dL	–	160 ± 91	173 ± 76	169 ± 62	> .05
BS2hpp, mg/dL	99 ± 15	223 ± 108	251 ± 117	235 ± 81	–
HbA1C (%)	–	8.1 ± 2	8.3 ± 2	8.1 ± 1	> .05
Serum Creatinine, mg/dL	0.8 ± 0.15	0.97 ± 0.18	0.99 ± 0.17	1 ± 0.16	> .05
e-GFR, mL/min/ 1.73m^2	94.7 ± 20	75 ± 15	76.8 ± 16	70 ± 9.2	> .05
Serum NGAL, ng/mL	174.9 ± 75.9	175.1 ± 117.8	219 ± 107.1	312 ± 150.9	< .001
Urine NGAL, ng/mL	67.8 ± 54.1	74 ± 83	72 ± 70.4	136.5 ± 139.6	> .05

Abbreviations: BS2hpp, blood sugar 2 hour post prandial; HbA1C, hemoglobin A1C; e-GFR, estimated glomerular filtration rate; NGAL, neurophil gelatinase-associated lipocalin
Statistical analysis: data are shown as the median (interquartile (IQR)) and frequency (%), for continuous and categorical variables, and in the same order, p-values are drawn from median and chi-squared tests.

microalbuminuric and macroalbuminuric states with the control group ($P > .05$, $P > .05$, and $P < .05$; respectively).

The correlation between u-NGAL and Alb/Cr ratio was significant for the diabetic participants and total population respectively (R = 0.17, $P < .05$; R = 0.18, $P < .05$).

A characteristic trend of increased u-NGAL in the macroalbuminuric group was seen, coinciding with the degree of the renal involvement (R = -0.34, $P < .05$).

By univariate regression analysis; serum Cr, urine Alb /Cr ratio and age were determined to be significant determinants of s-NGAL and also age, gender, urine Alb /Cr ratio, serum Cr, and e-GFR were found to be significant determinants of u-NGAL (Table 2).

In multivariate regression analysis of s-NGAL/u-NGAL, focusing on the diabetic participants, the micro and macroalbuminuric states were compared with normoalbuminuric as a reference group, in Table 3.

Based on this model, adjusting for the covariates (serum Cr, urine Alb /Cr ratio, and age), on average, the macroalbuminuric patients had higher levels of NGAL in their urine and serum compared with the normoalbuminuric group (the increments were 131.69 ng/mL and 66.11 ng/mL, respectively).

Comparison Between s- and u-NGAL Levels Among Control and Diabetic Subjects

As shown in Table 4, the normoalbuminuric patients and the control group had no significant differences in their s-NGAL, while its mean level was higher in the microalbuminuric patients than the two mentioned groups. Macroalbuminuric patients had significantly higher s-NGAL than other three groups. The mean level of u-NGAL showed no significant differences among the normoalbuminuric, microalbuminuric patients and the normal group, though the macroalbuminuric patients had significantly higher u-NGAL than other three groups. The mean differences of s-NGAL/u-NGAL are shown in Table 4 and 5.

Table 2. Univariate Regression Analyses Between s-NGAL/u-NGAL and Determinants Age, Diabetes Duration, Gender, Alb/Cr Ratio, Creatinine, and FBS (s-NGAL/u-NGAL in Upper and Lower Lines, Respectively)

s-NGAL u-NGAL	Regression Coefficient	95% CI	*P*
Age	1.78	0.53 to 3.03	< .01
	1.38	0.41 to 2.35	< .01
Diabetes Duration	0.62	-3.09 to 4.34	> .05
	0.28	-2.68 to 3.26	> .05
Gender (Male)	28.12	-4.76 to 61.01	> .05
	-30.60	-56.06 to -5.13	< .05
Alb/Cr Ratio	0.13	0.08 to 0.18	< .001
	0.05	0.01 to 0.09	< .01
Creatinine	164.4	79.10 to 249.70	< .001
	76.9	8.79 to 145.12	< .05
FBS	-0.19	-0.45 to 0.07	> .05
	-0.01	-0.22 to 0.19	> .05

Abbreviations: NGAL, neurophil gelatinase-associated lipocalin; Alb/Cr, albumin/creatinine, [b] FBS: Fasting Blood Glucose.

Table 3. Multivariate Regression Analysis Between s-NGAL/u-NGAL and State of Albuminuria, Adjusting for Age, Diabetes Duration, Gender, State of Albuminuria, and e-GFR.

s-NGAL u-NGAL	Coefficient	95% CI	*P*
Age	1.25	-1.22 to 3.72	> .05
	1.80	-0.23 to 3.83	> .05
Diabetes Duration	-1.27	-5.06 to 2.51	> .05
	-1.43	-4.54 to 1.67	> .05
Gender (Male)	21.87	-16.61 to 6.35	> .05
	-37.09	-68.68 to -5.51	< .05
State of Albuminuria			
Microalbuminuria	42.68	-1.60 to 86.98	> .05
	5.41	-30.94 to 41.76	> .05
Macroalbuminuria	131.69	82.53 to 180.85	< .001
	66.11	25.75 to 106.45	< .01
e-GFR	-0.38	-1.92 to 1.15	> .05
	-0.82	-2.10 to 0.44	> .05

Abbreviations: NGAL: neurophil gelatinase-associated lipocalin; e-GFR, estimated glomerular filtration rate.

Table 4. The Mean Difference of s-NGAL in Four Groups

State of Albuminuria	Mean Difference	Std. Error	*P*
Normal			
Normoalbuminuric	-0.61	21.28	> .05
Microalbuminuric	-44.66	20.53	< .05
Macroalbuminuric	-137.5	22.57	< .001
Normoalbuminuric			
Normal	-0.61	21.28	> .05
Microalbuminuric	-44.04	20.53	< .05
Macroalbuminuric	-136.96	20.57	< .001
Microalbuminuric			
Normal	44.66	20.53	< .05
Normoalbuminuric	44.07	20.53	< .05
Macroalbuminuric	-92.92	21.87	< .001
Macroalbuminuric			
Normal	137.58	22.57	< .001
Normoalbuminuric	136.96	22.57	< .001
Microalbuminuric	92.92	21.87	< .001

Table 5. The Mean Difference of u-NGAL in Four Groups

State of Albuminuria	Mean Difference	Std. Error	*P*
Normal			
Normoalbuminuric	0.27	0.21	> .05
Microalbuminuric	0.12	0.20	> .05
Macroalbuminuric	-0.55	0.22	< .05
Normoalbuminuric			
Normal	-0.27	0.21	> .05
Microalbuminuric	-0.15	0.20	> .05
Macroalbuminuric	-0.82	0.22	< .001
Microalbuminuric			
Normal	-0.12	0.20	> .05
Normoalbuminuric	0.15	0.20	> .05
Macroalbuminuric	-0.67	0.21	< .01
Macroalbuminuric			
Normal	0.55	0.22	< .05
Normoalbuminuric	0.82	0.22	< .001
Microalbuminuric	0.67	0.21	< .01

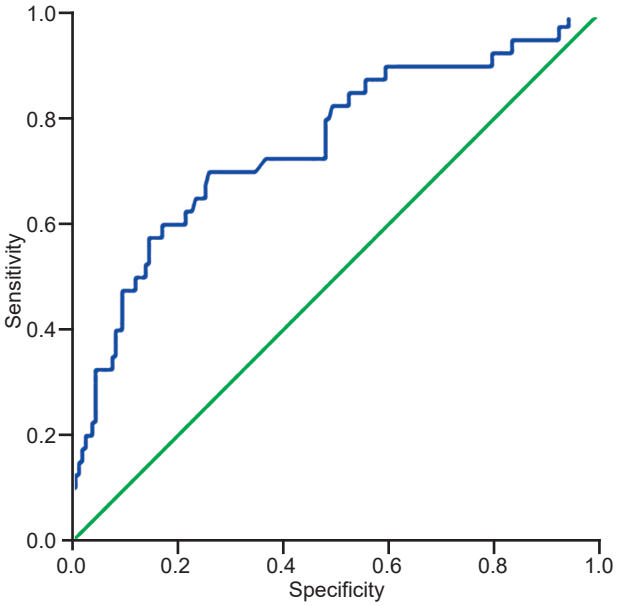

Figure 1. ROC Curve for the Macroalbuminuric State (as the Index Test) Comparing of Control Group (as the Reference Test) by s-NGAL (as a Predictor)

ROC Analysis of s-NGAL / u-NGAL

The area under curve (ROC) was calculated for comparing the three diabetic categories with the control group (s-NGAL / u-NGAL as a predictor), in the normoalbuminuric and microalbuminuric categories. No remarkable predictive powers were perceived in the normoalbuminuric categories, (s-NGAL: AUC = 0.48 and AUC = 0.46) and (u-NGAL: AUC = 0.46 and AUC = 0.47); respectively.

The ROC curve was plotted to detect the best sensitivity and specificity of s-NGAL/u-NGAL for the macroalbuminuric state, which shows 300 ng/mL and 71.4 ng/mL as the best target point for the detection of macroalbuminuric state, with the sensitivity of 26% and 60%, and the specificity of 98% and 72% respectively. In the macroalbuminuric group, the area under the curve (AUC) for the s-NGAL/u-NGAL was 0.60 and 0.67; respectively.

ROC analyses were performed to define the diagnostic outline of s-NGAL in identifying diabetic patients with macroalbuminuria. S-NGAL showed a good diagnostic outline, with an AUC of 0.75 and a best target value of 225.8 ng/mL (sensitivity = 70%, specificity = 74%).

For detecting the best target point of s-NGAL/u-NGAL in macroalbuminuric state the ROC curves were created and shown in Figures 1 and 2.

DISCUSSION

Diabetic patients are constantly exposed to hyperglycemia, hypertension and hyperlipidemia. These metabolic and hemodynamic stresses, result in inflammatory and atherosclerotic changes, which lead to endothelial dysfunction, and tubular damage in kidneys. Endothelial dysfunction leads to albuminuria while tubular damage, in turn, results in increase release of tubular biomarkers.[18,19] Therefore, diabetics, even with normal kidney function, are at increased risk for acute kidney injury compared with healthy individuals, due to diminished renal reserve, indicated by tubular markers such as NGAL and kidney injury molecule 1 (KIM-1), as clinical indicators of onset and progression of diabetic nephropathy.[20]

NGAL, a 25-kD a molecule, is a small protein which belongs to the superfamily of 'lipocalins', and is associated with purified gelatinase extracted from the supernatant of activated neutrophils.[21] Experimentally NGAL is overproduced by renal tubules within few hours of an ischemic or toxic injury (e.g. ischemia-reperfusion or cisplatin administration) which suggests that this biomarker is involved in pathogenesis of stress-induced acute renal damage.[22-26]

In practice, therefore, measurement of serum or urine NGAL can be used as an early biomarker in acute kidney injury following the use of nephrotoxic agents such as contrast agents or in cardiac surgery, as NGAL level rises in predisposed individuals

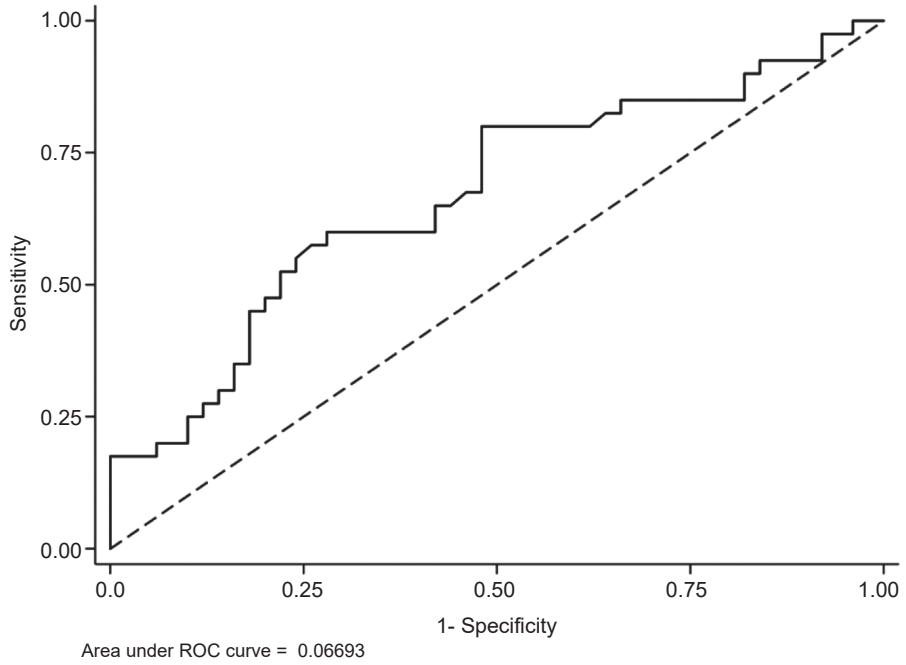

Figure 2. ROC Curve for the Macroalbuminuric State (as the Index Test) Comparing of Control Group (as the Reference Test) by u-NGAL (as a Predictor)
No adverse events were reported from performing the index tests or the reference standard evaluation.

preceding the increase in serum creatinine.[17,23-26]

In the present study we aimed to evaluate the level of serum and urine NGAL, in type 2 diabetic patients at different stages of albuminuria as a predictive factor of progression of diabetic nephropathy and thereby contributing to preventive and treatment options in proper time.

The result of our study revealed that all diabetic patients with albuminuria had elevated s-NGAL values compared with diabetics without albuminuria (normoalbuminurics) and healthy control group. Additionally, a significant drift was observed in macroalbuminuric state, in a way that s-NGAL values increased in parallel with increasing severity of albuminuria and renal involvement, approaching higher levels in patients with overt proteinuria ($P < .001$).

These findings are similar to the study conducted by Nielsen *et al.* on 58 normoalbuminuric, 45 microalbuminuric and 45 macroalbuminuric type 1 diabetic patients and 55 non-diabetic control subjects, in which comparable trends for s-NGAL and other biomarkers of tubular damage, such as KIM-1 have been reported, which increased levels were found to be proportional to the degree of urinary albumin excretion.[14] These also support the developing theory of a 'tubular phase' of diabetic disease that heralds or coincides with the manifestation of classic glomerular lesions.[20]

Although we couldn't show statistically significant increase in s-NGAL in normoalbuminuric group in the current study, it was found that s-NGAL increases with the appearance of albuminuria and it could be claimed that s-NGAL has a sensitivity of 98% for diagnosis of microalbuminuria.

On the other hand, another study which was performed by Bolignano *et al.* on fifty-six patients with type 2 insulin dependent diabetes mellitus and 18 healthy controls, showed that all diabetic patients presented with elevated s-NGAL values compared with healthy control group and s-NGAL level increases even before the appearance of albuminuria in diabetic patients, which could be used as an early measurable marker of renal involvement in diabetic patients, although this study revealed the similar results of s-NGAL increment in micro- or macro- albuminurics as our study [6], we couldn't show increase in s-NGAL level in diabetics without albuminuria.

In another recently published study which was performed on 144 type 2 diabetic individuals and 54 control populations, the s-NGAL was significantly higher in diabetic individuals as compared with the control group by means of

significant difference between the groups ($P < 0.05$). They also showed significant difference in s-NGAL between normoalbuminuric diabetic patients and control healthy population ($P < .05$). This study revealed that tubular injury might occur in advance of glomerular injury in diabetic individuals, and s-NGAL can be used as a biomarker to detect diabetic nephropathy even before overt nephropathy and also be used as a noninvasive test for diagnosis, staging, and progression of DN.[18] Although the result of this study was somewhat different from our study, as we couldn't show significant increase in s-NGAL in normoalbuminuric group, they both showed correlation of this biomarker with the development and progression of albuminuria.

The u-NGAL and Alb/Cr ratio were significantly correlated in both the diabetic participants and normal population. In the macroalbuminuric group, an increased trend of u-NGAL was seen in parallel with the severity of the renal involvement, therefore we could also suggest u-NGAL as a probable predictor of macroalbuminuria.

By univariate regression analysis; e-GFR, Cr, Alb /Cr ratio, age and gender were found to be significant determinants of u-NGAL. Woo *et al.* concluded in their study that u-NGAL is a reliable marker of renal function in diabetic patients with CKD. They showed that urinary NGAL level has significant inverse correlation with GFR ($P < .0001$). However, urinary NGAL did not provide more accurate information regarding renal function than GFR in their study.[8] This is consistent with our study that showed direct correlation of Alb/Cr and inverse correlation of Cr, e-GFR ratio with u-NGAL.

Based on the multivariate regression model, the mean level of u-NGAL of the patients in the macroalbuminuric was found to be 66.11 ng/mL more than the normoalbuminuric group.

The best sensitivity, specificity cut-off point of u-NGAL for the macroalbuminuric state, was 71.4 ng/mL for detection of macroalbuminuric state. The sensitivity was 60% and the specificity was 72%, which could define the u-NGAL as a poor predictor of macroalbuminuria level.

Many clinical studies have demonstrated the utility of u-NGAL as an earlier, non-invasive, specific, and appropriate predictor of renal involvement in the progression of diabetes, acute kidney injury and CKD.[17,27-29]

In the study which was performed by Agnieszka Zylka *et al.* on 55 Type 2 Diabetic patients, they concluded that first morning u-NGAL and u-NGAL/Creatinine may be considered useful to evaluate renal function and cardiovascular risk, accompanied by albuminuria and eGFR.[30] In our study we couldn't show increased u-NGAL in diabetics with microalbuminuria while increased level was found in those with normal Alb/Cr and control group. It seems that urine concentration affects the urinary level of NGAL and it is better to collect early morning urine sample to increase the accuracy of the results.

The present study revealed highly increment in u-NGAL levels in macroalbuminuric state in comparison with control group. These results were consistent with previous reports which indicate a high mean u-NGAL concentration in subjects with CKD (non-diabetic) compared with healthy group,[17] the u-NGAL levels were significantly elevated in diabetic type 2 CKD patients compared with the normal subjects.[8]

We also observed increased levels of u-NGAL in normoalbuminuric diabetics compared with microalbuminuric group. The result of our cross-sectional study indicates that the tubular damage, expressed by u-NGAL is present before the development of microalbuminuria which is in agreement of other study done by Nielsen that their study included type 1 diabetic patients with overt nephropathy.[14,31]

Finally in the study which was conducted by Matys *et al.*, 121 patients with diabetes who were candidate for coronary angiography were included, u-NGAL, cystatin C and KIM-1 were not more useful than eGFR in the assessment of kidney function in diabetic patients with coronary heart disease.[32] Since u-NGAL can be affected by many factors such as urine concentration and e-GFR, it might be less useful in prediction of kidney function in diabetic patients.

CONCLUSION

Serum and urine-NGAL is elevated in type 2 diabetic patients, with or without albuminuria, s-NGAL level clearly correlates with severity of renal damage caused by DN and u-NGAL especially increased by macroalbuminuric state. Finally, s-NGAL measurement could be a useful, noninvasive, easily available and practical test for

the evaluation of severity of renal involvement in the course of diabetes. We suggest u-NGAL as a probable predictor of macroalbuminuria. It is also recommended to perform more detailed studies to approve the possible usefulness of s-NGAL as a biomarker for the early diagnosis of diabetic nephropathy and to confirm its ultimate role for monitoring the development and progression of diabetic nephropathy in the future. And also future investigations in different clinical settings with larger sample size are necessary to explicate the efficacy and cost-effectiveness of u-NGAL in determining renal function and disease progression.

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