Urinary neutrophil gelatinase-associated lipocalin in early detection of diabetic nephropathy; a pilot study

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ABSTRACT

Introduction: Diabetes results in the high prevalence of diabetes and the subsequent high incidence of nephropathy. However, there is no method with high sensitivity for the early detection of diabetic kidney disease.

Objectives: The objective of this study was to evaluate the effect of the frequency of neutrophil gelatinase-associated lipocalin (NGAL) in the early diagnosis of diabetic nephropathy.

Patients and Methods: Seventy-six diabetic patients who referred to the diabetes clinic of Tohid hospital were randomly selected and enrolled into this cross-sectional study. Patients with hypertension, primary and secondary glomerulopathy, every type of malignancy, infection, heart disease, pulmonary disease and other endocrine diseases, and significant renal failure (estimated glomerular filtration rate; eGFR≤30 mL/min) were excluded from the study. Demographic data were collected and the patients were divided into normal and abnormal groups based on the results of urine tests. Morning urine samples were then taken from the patients to measure creatinine, albumin, urine Alb/CR (albumin to creatinine) ratio and NGAL.

Results: Of a total of 76 patients who were enrolled in the study, 39 persons (51.3%) were female. The mean age of the participants was 59.03 ± 11.74 years. In addition, 64 patients (84.2%) were in the normoalbuminuria group and 12 persons (15.8%) were in the microalbuminuria group. Urinary NGAL level was significantly higher in the microalbuminuria group than in the normal group since the difference was significant ($P < 0.001$). We found urinary NGAL level had no significant relationship with eGFR value and the duration of diabetes ($P > 0.05$). There was also a relationship between urinary NGAL and urinary Alb/CR ratio levels ($P = 0.001$).

Conclusion: The results of the present study showed a relationship between urinary NGAL and urine Alb/CR ratio levels which could be used as a suitable biomarker in the diagnosis of diabetic nephropathy. Moreover, urinary NGAL level had no significant relationship with eGFR and the duration of diabetes.

Original

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Introduction

Diabetes is a chronic metabolic disease characterized by an increase in blood sugar and disorders in carbohydrate, fat, and protein metabolism. It is one of the most important health, medical, social, and economic problems in the world today (1). According to published data by the World Health Organization (WHO), the global prevalence of different types of diabetes is 422 million, and it has an increasing trend (2).

Diabetic nephropathy (diabetic kidney disease) is the most common microvascular complication of diabetes mellitus and is the most common cause of renal failure, which occurs in 25%-30% of patients with type 2 diabetes (3). Pathologically, there is the involvement of glomerular endothelial and tubular epithelial and interstitial cells (4). Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) and premature death in diabetic patients worldwide. It not only causes progressive renal failure but also leads to mortality due to cardiovascular diseases in diabetic patients. In addition, the majority of patients with this disease die from complications of atherosclerotic disease rather than renal failure. Hence, early detection of this disease is of great importance (5).

Diabetic kidney disease is diagnosed by measuring serum creatinine levels and calculating urinary albumin to creatinine ratio or albuminuria level, while they have not enough sensitivity for early detection (6). Although various markers have been suggested as the early predictors of renal impairment in diabetic patients, their precise accuracy has not been established yet. Hence, microalbuminuria is considered as a marker of diabetic nephropathy for the initiation of treatment in diabetic patients (7). Neutrophil gelatinase-associated lipocalin (NGAL) is one of these markers. NGAL is a small, 25 kDa protein from the lipocalin family, which is associated with the matrix metalloproteinase 9 that carries iron and is stored as granules in neutrophils. However, to a lesser extent, it is observed in several human tissues such as kidney, trachea, lung, stomach, and colon and plays an important role in innate immunity and bacterial infections (8). The expression of NGAL in ischemic and toxic kidney injuries is increased at the surface of renal tubules and is secreted in plasma and urine. NGAL levels increase in plasma and urine two hours after renal injury, thus urine and plasma NGAL levels can be used as a sensitive marker for early diagnosis of renal failure (9). Various studies have shown the role of NGAL in the diagnosis of diabetic nephropathy (10).

Objectives

The high prevalence of diabetes and, consequently, the high incidence of diabetic kidney disease and lack of high sensitivity and practical method for early detection had led us to investigate the use of NGAL for the early diagnosis of diabetic nephropathy. This study aimed to assess NGAL, in early diagnosis of diabetic nephropathy.

Patients and Methods

Study patient

In this cross-sectional study, 76 diabetic patients who referred to the diabetes clinic of Tohid hospital were randomly selected (2018). Patients with hypertension, primary or secondary glomerulopathy, every type of malignancy, infection, heart disease, pulmonary disease and other endocrine diseases, and significant renal failure (estimated glomerular filtration rate; eGFR ≤30 mL/min) were excluded from the study. Demographic data including age, gender, and duration of diabetes were collected by a researcher-made checklist since the ethical issues were observed. Urine samples were taken in the morning to measure creatinine, albumin, urine Alb/Cr (albumin to creatinine) ratio and NGAL. Then based on the results of Alb/Cr urine test, patients were divided into two groups, including normoalbuminuria group (<30 mg/g) and microalbuminuria group (>300 mg/g). The human NGAL Elisa kit (Shanghai crystal Day Biotech CO, LTD, E1719Hu) was used to measure urinary NGAL. Glomerular filtration rate (GFR) was estimated by an equation developed by the chronic kidney disease epidemiology (CKD-EPI) collaboration. Moreover, the following biochemical tests were performed for this study; serum creatinine, urine creatinine, and urine albumin test (10).

Ethical issues

The research followed the tenets of the Declaration of Helsinki and its later amendments. This study was approved by the Ethics Committee of the Kurdistan University of Medical Sciences (IR.MUK.REC.1397.168). All procedures performed in studies involving human participants were in accordance with the ethical guidelines of the institutional research committee. All participants provided written and informed consent. Additionally, this study was extracted from the internal medicine residency thesis of Fowzieh Khezrian at this university.

Statistical analysis

Frequency and percentage of variables were calculated and *t* test was used to compare the variables. In this study, the collected data were analyzed using SPSS 22 software. The significance level was set at $P < 0.05$.

Results

Out of 76 studied patients, 39 persons (51.3%) were female. The mean age of the participants was 59.03 ± 11.74 years. The mean duration of diabetes was 12.2 ± 5.02 years. Around, 64 persons (84.2%) were in the normoalbuminuria group since 12 persons (15.8%) were

Table 1. Comparison of demographic and clinical characteristics in people with diabetes

	Normoalbuminuria (n=64, 84.2%)	Microalbuminuria (n=12, 15.8%)	P value
Gender (male/ female)	35.30	4.7	0.283
Age (y)	59.1 (11.53)	57.67 (12.85)	0.909
Weight (kg)	73.57 (10.54)	76.08 (13.52)	0.493
Duration of diabetes (y)	12.20 (4.99)	10.50 (5.19)	0.853
Urine NGAL test (ng/mL)	70 (17.04)	99.77 (43.56)	<0.001
eGFR (ml/ min/1.73 m²)	75.85 (21.26)	77.05 (34.14)	0.873
Alb/CR (urine) (mg/g)	0.010 (0.007)	0.084 (0.074)	<0.001

Figure 1. Relationship between NGAL and albumin to creatinine ratio.

in the microalbuminuria group (Table 1).

The results showed that the ratio of males to females, mean age, weight, and duration of diabetes, serum creatinine and estimated glomerular filtration rate (eGFR) level did not show any significant difference between two groups (P>0.05).

The results of this study also showed that the level of urinary NGAL test in the microalbuminuria group was higher than that in the normoalbuminuria group (P<0.001). There was also a significant correlation between NGAL test and urinary Alb/CR ratio (P=0.001; Figure 1). However, there was no statistically significant difference between two groups with eGFR <60 mL/min/1.73 m² and eGFR ≥60 mL/min/1.73m² regarding the urinary NGAL level (P=0.958; Table 1).

Discussion

One of the most important and most serious complications of diabetes is diabetic kidney disease which has different stages. Diabetic nephropathy is the most common microvascular complication and the most common cause of renal failure. Serum creatinine is a non-sensitive marker that can indicate the presence of acute renal injury, though with a delay. Therefore, other diagnostic markers are required for the early diagnosis of acute renal injury. Because of its significance, an increasing body of research is conducted to identify new biomarkers for the diagnosis of acute renal injury. Among the likely markers, NGAL is the most potential molecule among the other molecules. Mishra et al identified this marker as an effective biomarker for the early diagnosis of acute renal injury. Accordingly, various clinical studies have been performed to date in this field.

The results of our study showed that the level of urinary Alb/CR ratio was lower in the normoalbuminuria group than in the microalbuminuria group and there was a significant difference between the two groups (P=0.005).
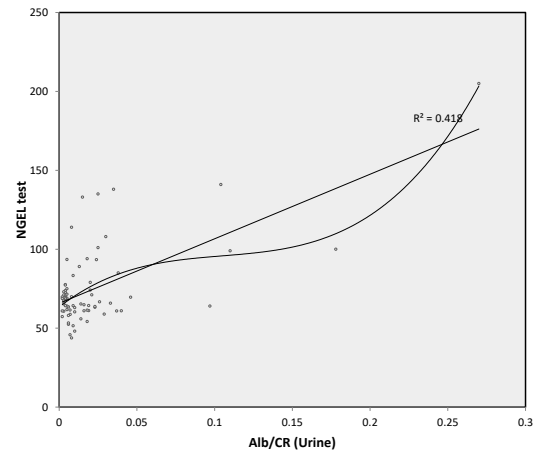
The results also showed a significant correlation between NGAL test and urinary Alb/CR ratio (P=0.001). In a study by Siddiqi et al, the ratio of albumin to urinary creatinine level in microalbuminuria group was significantly higher than that in normoalbuminuria group while there was also a significant positive correlation between serum and urine NGAL level with urinary Alb/CR ratio, which is in agreement with the results of our study (10). In the study by Hafez et al, a significant positive correlation between urine Alb/CR ratio and urinary NGAL levels in microalbuminuria patients was detected, indicating that renal disease was more severe in their patients that consequently increased urinary NGAL level (7).

The results of our study showed that NGAL level was higher in the microalbuminuria group than in the normoalbuminuria group since the difference was also statistically significant. Consistent with the results of our study, in the study by Siddiqi et al, serum and urine NGAL levels in microalbuminuria patients were higher than the control group (normoalbuminuria) while this difference was statistically significant too(10). In a study by Bolignano et al, urinary NGAL and urinary albumin levels in diabetic patients were higher than those in the control group with a statistically significant difference (11). In addition, Nauta et al showed that the level of NGAL in diabetic patients with normoalbuminuria was higher than that in the control group (non-diabetic patients) (12). Accordingly, the results of the study by Nielsen et al showed that increased urinary NGAL was associated with increased albuminuria since this tubular protein was significantly higher in macro and microalbuminuria groups than in the normoalbuminuria group. They also showed that urinary NGAL was higher in the normoalbuminuria group than in the control group (13). As a result, it can be used as a suitable biomarker for the diagnosis of diabetic nephropathy in patients with microalbuminuria. In this study, results of serum

ceratinine and eGFR had no significant difference between two groups, regarding normoalbuminuria and microalbuminuria groups ($P > 0.05$). The results of our study also showed no significant difference between the two groups with eGFR ≥ 60 mL/min/1.73 m^2 and eGFR < 60 mL/min/1.73 m^2 ($P = 0.958$) in terms of NAGAL. Recently, Seibert et al showed no significant correlation between NGAL and eGFR in patients with stable chronic kidney disease, including those with diabetes, hypertensive nephropathy, glomerulonephritis/vasculitis and autosomal dominant polycystic kidney disease (14), while in the study by Maty et al, a negative significant correlation between NAGAL and eGFR < 60 mL/min/1.73 m^2 (r=-0.480, $P < 0.001$) was found (15). In contrast with the results of our study, the results of the study by Kaul et al showed a statistically significant difference between the four groups (control, normoalbuminuria, micro and macroalbuminuria) regarding eGFR levels ($P < 0.001$) (16). In our study, the patients who were selected randomly may have had a high eGFR and had been in the early stages of diabetic kidney disease.

Conclusion
Urinary NGAL has a relationship with urine Alb/CR ratio and can, therefore, be used as a suitable biomarker for the diagnosis of diabetic nephropathy.

Limitations of the study
This study was conducted on a limited number of patients and it is recommended to conduct additional studies on this subject (especially nested case–control study) on larger samples.

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Authors' contribution
FK, MSF, LS and SG designed the study. FK and SK collected the data. KR and DR finalized analysis and refined data. FK and MSF supervised the study. PS and ZR wrote the paper. All authors read, revised and approved the final manuscript.

Conflicts of interest
The author declared no conflict of interests.

Ethical considerations
Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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References
1. Uwaezuoke S. Prevention of diabetic nephropathy in children and adolescents: how effective are the current strategies. Int J Diabetol Vasc Dis Res. 2015;5:1-5.
2. Ruiz-Ortega M, Rayego-Mateos S, Lamas S, Ortiz A, Rodrigues-Diez RR. Targeting the progression of chronic kidney disease. Nat Rev Nephrol. 2020 Feb 14. doi: 10.1038/s41581-019-0248-y.
3. Donate-Correa J, Luis-Rodríguez D, Martín-Núñez E, Tagua VG, Hernández-Carballo C, Ferri C, Rodríguez-Rodríguez AE, et al. Inflammatory Targets in Diabetic Nephropathy. J Clin Med. 2020;9:458. doi: 10.3390/jcm9020458.
4. Chen B, Li Y, Liu Y, Zang C, Wu M, Xu Z. Diagnostic value of neutrophil gelatinase-associated lipocalin in diabetic nephropathy: a meta-analysis. Ren Fail. 2019;41:489-96. doi: 10.1080/0886022X.2019.1619581.
5. Fu H, Liu S, Bastacky SI, Wang X, Tian XJ, Zhou D. Diabetic kidney diseases revisited: a new perspective for a new era. Mol Metab. 2019;30:250-263. doi: 10.1016/j.molmet.2019.
6. Lee S-Y, Choi ME. Urinary biomarkers for early diabetic nephropathy: beyond albuminuria. Pediatr Nephrol. 2015;30:1063-75. doi: 10.1007/s00467-014-2888-2
7. Hafez MH, El-Mougy FA, Makar SH, El Shaheed SSA. Detection of an earlier tubulopathy in diabetic nephropathy among children with normoalbuminuria. Iran J Kidney Dis. 2015;9:126.
8. Vijay S, Hamide A, Senthilkumar GP, Mehalingam V. Utility of urinary biomarkers as a diagnostic tool for early diabetic nephropathy in patients with type 2 diabetes mellitus. Diabetes Metab Syndr. 2018;12:649-52. doi: 10.1016/j.dsx.2018.04.017.
9. Motawi TK, Shehata NI, ElNokeety MM, El-Emady YF. Potential serum biomarkers for early detection of diabetic nephropathy. Diabetes Res Clin Pract. 2018;136:150-8. doi: 10.1016/j.diabres.2017.12.007.
10. Siddiqi Z, Karoli R, Kaul A, Fatima J, Varshney S, Beg MS. Evaluation of neutrophil gelatinase-associated lipocalin and cystatin C as early markers of diabetic nephropathy. Ann. Afr. Med. 2017;16:101. doi: 10.4103/aam.aam_12_17.
11. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol. 2009;4:337-44. doi: 10.2215/CJN.03530708.
12. Nauta FL, Boertien WE, Bakker SJ, Van Goor H, Van Oeveren W, De Jong PE, et al. Glomerular and tubular damage markers are elevated in patients with diabetes. Diabetes Care. 2011;34:975-81. doi: 10.2337/dc10-1545.
13. Nielsen S, Schjoedt K, Astrup A, Tarnow L, Lajer M, Hansen P, et al. Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Kidney Injury Molecule 1 (KIM1) in patients with diabetic nephropathy: a cross-sectional study and the effects of lisinopril. Diabet Med. 2010;27:1144-50. doi: 10.1111/j.1464-5491.2010.03083.x.
14. Seibert FS, Sitz M, Passfall J, Haesner M, Laschinski P, Buhl

M, et al. Prognostic value of urinary calprotectin, NGAL and KIM-1 in chronic kidney disease. Kidney Blood Press Res. 2018;43:1255-62. doi: 10.1159/000492407.

15. Matys U, Bachorzewska-Gajewska H, Malyszko J, Dobrzycki S. Assessment of kidney function in diabetic patients. Is there a role for new biomarkers NGAL, cystatin C and KIM-1? Adv Med Sci. 2013;58:353-61. doi: 10.2478/v10039-012-0077-8

16. Kaul A, Behera M, Rai M, Mishra P, Bhaduaria D, Yadav S, et al. Neutrophil gelatinase-associated lipocalin: as a predictor of early diabetic nephropathy in type 2 diabetes mellitus. Indian J Nephrol. 2018;28:53. doi: 10.4103/ijn. IJN_96_17.