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Detection of an Earlier Tubulopathy in Diabetic Nephropathy Among Children With Normoalbuminuria

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Introduction. Diabetic nephropathy is a major cause of morbidity and mortality among young adults with type 1 diabetes mellitus (DM). Albuminuria, the gold standard for early diagnosis, cannot always detect early diabetic nephropathy. We aimed at evaluating the level of urine neutrophil gelatinase-associated lipocalin (NGAL) as a marker of tubulointerstitial damage in children and adolescents with type 1 DM in relation to the level of albuminuria and other parameters.

Materials and Methods. Fifty children with type 1 DM for more than 5 years were included in this study (mean age, 13.8 ± 4.0 years), and 18 healthy children served as controls. Patients with overt albuminuria (> 300 mg/g creatinine) or inflammatory states were excluded. Urine NGAL, microalbuminuria, and urine albumin-creatinine ratio were measured in patients and controls as well as other parameters.

Results. Urine NGAL was significantly higher in microalbuminuric in comparison with normoalbuminuric patients and controls, and correlated positively with urine albumin-creatinine ratio. A positive urine NGAL was observed in 12 of 38 normoalbuminuric patients (31.6%) compared to 9 of 12 microalbuminuric patients (75%). A positive correlation was reported between urine NGAL and both Hemoglobin A1c and duration of DM, but not with estimated glomerular filtration rate or hypertension.

Conclusions. Diabetic children, even some normoalbuminurics, showed increased urine NGAL. This finding may support the hypothesis of a "tubular phase" of diabetic disease preceding overt diabetic nephropathy, and hence, the use of urine NGAL measurement for early evaluation of renal involvement.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple causes characterized by chronic hyperglycemia and disorders of carbohydrate, fat, and protein metabolism. It may be classified as autoimmune mediated type 1 DM, or as insulin resistance-associated type 2 DM, or a combination of these factors. Type 1 DM commonly occurs in childhood or adolescence, although the rising prevalence of type 2 DM in these age groups is now being seen worldwide.[1]

Diabetic nephropathy (DN) is one of the most common microvascular complications of DM, greatly affecting the life quality and survival of the patients. In adults, DN is one of the leading causes of end-stage renal disease (ESRD).[2] The prevention

of the disease or at least the postponement of its progression has emerged as a key issue. Adverse outcomes of kidney failure can be prevented or delayed through early detection and treatment.[3]

At present, albuminuria measurement is used as a standardized noninvasive test to diagnose early DN. Diabetic kidney disease, however, is not detected by this test in some cases.[4] Pathological albuminuria and proteinuria constitute the consequence of diffuse diabetes-induced glomerular damage. However, renal tubule-interstitium also seems to play an equally important role in the genesis of DN, as the consequence of a persistent exposure to a variety of metabolic and hemodynamic injuring factors associated with sustained diabetic disease.[5]

Neutrophil gelatinase-associated lipocalin (NGAL) is an acute-phase protein that is rapidly released not only from neutrophils, but also a variety of cell types upon inflammation and tissue injury. Its small molecular size and protease resistance could render it an excellent biomarker of kidney injury.[6] Circulating NGAL is filtered by the glomerulus and captured by the proximal tubule and only a minimal amount is excreted in urine.[7] In contrast, urine NGAL derives mostly from the thick limbs of the Henle and collecting ducts in both the postischemic and postseptic kidney. Its values in children and adults are markedly elevated with acute kidney injury, anticipating the rise of creatinine by 24 to 48 hours.[7]

Neutrophil gelatinase-associated lipocalin might play an important role in the pathophysiology of renal adaptation to DM, probably as a defensive mechanism aiming to mitigate tubular suffering. Furthermore, urine NGAL measurement might become a useful and noninvasive tool for evaluation of renal involvement in diabetic patients in its incipient phase, even earlier than the established phase of DN assessed classically by microalbuminuria.[5] We aimed to evaluate the level of urine NGAL as a marker of tubulointerstitial damage in children and adolescents with type 1 DM in relation to the level of albuminuria and other parameters.

MATERIALS AND METHODS

Study Population

This study included 50 children and adolescents with type 1 DM for more than 5 years' duration. Patients were recruited from regular attendance of the Diabetic Endocrine Metabolic Unit, Children Hospital, Cairo University, Egypt. Microalbuminuria was measured in the 50 patients included in the study group who were further divided into group 1, consisting of 38 normoalbuminuric patients (< 30 mg/g creatinine) and group 2, consisting of 12 microalbuminuric patients (30 mg/g to 300 mg/g). Patients enrolled in the study were not on angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers at the time of sampling. Those who were on ACEI or angiotensin receptor blockers were required to stop that 1 week before the study, and in case of hypertension, they were shifted to amlodipine or a beta-blocker. Exclusion criteria included those with overt albuminuria (> 300 mg/g creatinine) or inflammatory states. Eighteen healthy age- and sex-matched children served as controls, with a mean age of 12.17 ± 4.32 years.

Methods

All of the patients were subjected to a detailed history including onset of the disease, frequency of diabetic ketoacidosis or hypoglycemia as well as insulin therapy (type, basal-bolus ratio, dose, and frequency). Data on any history suggestive of complications was collected from patients' charts, including ocular, cardiac, neurological, dermatological, or other associated diseases such as autoimmune thyroiditis, Addison disease, and celiac disease. Special focus on renal complications including hypertension and microalbuminuria was noted. A thorough physical examination was done for all of the patients and controls. In all of the patients, the following blood investigations were done: serum creatinine and estimated glomerular filtration rate (GFR) calculated by Schwartz formula (GFR = k × height (cm)/serum creatinine (mg/dL), where k was equal to 0.55 in children aged 5 to 13 years, 0.7 in adolescent males and 0.55 for adolescent females[4,8]; lipid profile including total cholesterol, serum high-density lipoprotein cholesterol, and triglycerides concentrations, determined enzymatically using commercially available kits on autoanalyzer (Olympus AU 400, USA), as well as low-density lipoprotein cholesterol, estimated using Friedewald formula[9]; thyroid profile including free T4 measured with radioimunonoassay methods, serum thyroid-stimulating hormone with immunoradiometric assay

method by commercial kits (Diagnostic Products Corporation, Los Angeles, USA); and mean values of last 3 glycosylated hemoglobin A1c (HbA1c) in the last 9 months prior to the study with quantitative colorimetric determination of glycohemoglobin in whole blood with cation-exchange resin (Stanbio Laboratory, Texas, USA).[10] Urine examinations included complete urinalysis; albumin-creatinine ratio in the first morning urine sample; persistent microalbuminuria (first morning sample) confirmed by 3 successful albumin-creatinine ratios more than 30 mg/g, on 3 separate occasions (assessment of microalbuminuria was done in the absence of confounders namely urinary tract infections, exercise, and menstrual bleeding. Microalbuminuria was measured by immunonephelometric method on Prospec Siemens, Siemens Healthcare Diagnostic Inc, Newark, USA)[11]; and urine NGAL (which was done for controls as well as for patients).

Written informed consent was taken from patients' care providers prior to the study. The current study agrees with the Declaration of Helsinki and its revisions and it was approved by the Committee on Human Experimentation in the Center of Pediatric Nephrology and Transplantation and the Department of Pediatric Endocrinology, Cairo University Children Hospital, and received the approval of the research and scientific committee of the Department of General Pediatrics, Cairo University.

Statistical Analyses

The SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA) was used for analysis of data. Data were summarized as mean ± standard deviation. The nonparametric Mann-Whitney U test was used for analysis of quantitative data. The chi-square test was used for analysis of qualitative data. The 1-way analysis of variance test was used for analysis of more than 2 groups followed by post HOCC test for detection of significance. The Spearman rank correlation was also used for analysis of correlations, and a coefficient was considered weak if less than 0.25, mild if between 0.25 and 0.49, moderate if between 0.5 and 0.74, and strong if greater than 0.75. A receiver operating characteristic curve analysis was done to identify the optimal cutoff of urine NGAL for detection of microalbuminuria. *P* values less than .05 were considered significant.

RESULTS

The present cross-sectional study included 50 patients with type 1 DM with a mean age of 13.8 ± 4.0 years. All of the patients included had a duration of DM more than 5 years with a mean duration of 8.57 ± 0.53 years. Sixteen patients (32%) were boys. Demographic, anthropometric, clinical, and laboratory data of the patients with type 1 DM are shown in Table 1. A sample of 18 healthy children and adolescents with a mean age of 12.2 ± 4.3 years were included in the study as controls, 7 of whom (38.9%) were boys.

The mean urine NGAL level in the patients was 21.32 ± 27.80 ng/mL (range, 0.4 ng/mL to 100 ng/mL), which was significantly higher when compared with the controls (mean, 5.66 ± 5.08 ng/mL; range, 0.25 ng/mL to 15.8 ng/mL; P = .02). Further subdivision of patients yielded

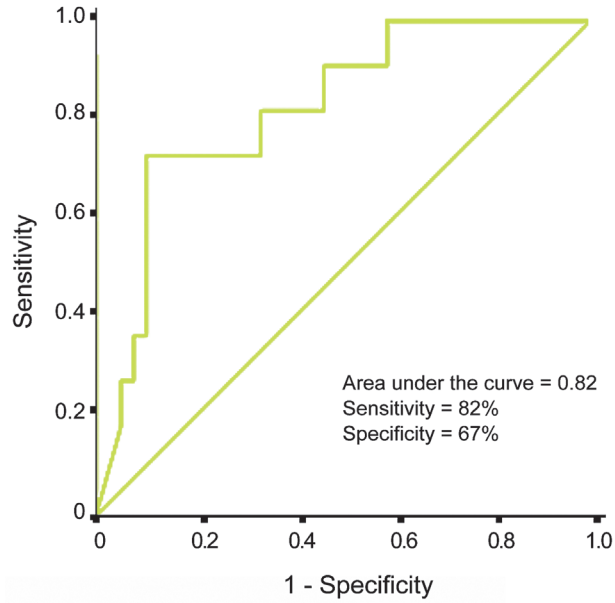
Table 1. Descriptive Statistics of Patients Included in the Study (n = 50)*

Parameter	Mean Value (Range)
Demographic data	
Age, y	13.84 ± 4.00 (6.1 to 18.0)
Duration of disease, y	8.57 ± 0.53 (5.0 to 17.5)
Insulin dose, IU/kg/d	1.16 ± 0.44 (0.4 to 2.3)
Anthropometric data	
Weight, kg	47.88 ± 18.08 (15.5 to 97.0)
Weight, SDS	0.28 ± 1.49 (-2.44 to 3.89)
Height, cm	56.18 ± 71.21 (112.0 to 161.5)
Height, SDS	-1.05 ± 1.38 (-3.47 to 2.60)
BMI, kg/m^2	22.19 ± 5.79 (14.17 to 40.10)
BMI, SDS	0.75 ± 1.39 (-3.67 to 3.10)
Clinical data	
Systolic blood pressure, mm Hg	112.7 ± 15.0 (85 to 150)
Diastolic blood pressure, mm Hg	72.9 ± 12.2 (50 to 110)
Laboratory data	
Urine albumin-creatinine, mg/g	34.75 ± 51.71 (4.0 to 280.0)
Creatinine, mg/dL	0.67 ± 0.16 (0.3 to 1.2)
Estimated GFR, mL/mim/m^2	133.53 ± 38.90 (67.8 to 278.6)
Hemoglobin A1c, %	8.29 ± 1.29 (5.9 to 10.8)
Serum HDLC, mg/dL	51.96 ± 15.60 (14.0 to 86.0)
Serum LDLC, mg/dL	116.61 ± 33.81 (49.0 to 217.0)
Serum total cholesterol, mg/dL	174.18 ± 40.77 (96.0 to 327.0)
Serum triglycerides, mg/dL	66.19 ± 37.57 (21.0 to 244.0)
Urine NGAL, ng/mL	21.32 ± 27.80 (0.4 to 100.0)†

*SDS indicates standard deviation score; BMI, body mass index; GFR, glomerular filtration rate; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; and NGAL, neutrophil gelatinase-associated lipocalin.
†Median of urine NGAL was 10.15 ng/mL.

2 groups of normoalbuminuric group (n = 38) with a mean urine NGAL of 15.69 ± 23.25 ng/mL (median, 5.7 ng/mL; range, 0.4 ng/mL to 100 ng/mL) and microalbuminuric group (n = 12) with a mean urine NGAL of 39.14 ± 33.98 ng/mL (median, 31.25 ng/mL; range, 2.7 ng/mL to 100 ng/mL). Comparison of the normoalbuminuric and microalbuminuric groups with controls is illustrated in Table 2. Urine NGAL level was significantly higher in microalbuminuric patients compared to both normoalbuminuric patients and controls (P = .001), and although its level was higher in normoalbuminuric patients compared to the controls, this difference was not significant.

The receiver operating characteristic curve for detection of microalbuminuria by urine NGAL is illustrated in the Figure. The area under the curve was 0.821. The cutoff level for NGAL with the best sensitivity and specificity was measured to be 11.75 ng/mL, with a sensitivity of 82% and a specificity of 67%.



Receiver operating characteristic plot analysis of urine neutrophil gelatinase-associated lipocalin in normoalbumuinuric and microalbumuinuric patients.

DISCUSSION

In the current study, as well as in other studies,[3,4,12,13] urine NGAL was significantly higher in all diabetic children in comparison to the controls. Bolignano and colleagues reported that the mean urine NGAL values in their microalbuminuric group were significantly high compared with controls and normoalbuminuric patients.[5] Also, Fu

Table 2. Comparisons Between Normoalbuminuric and Microalbuminuric Patients and Controls

Parameter	Normoalbuminuric Patients (n = 38)	Microalbuminuric Patients (n = 12)	Controls (n = 18)	*P*
Systolic blood pressure, mm Hg	110.00 ± 13	121.25 ± 18.11	111.39 ± 7.63	.04
Diastolic blood pressure, mm Hg	70.79 ± 11.18	79.58 ± 13.39	74.17 ± 8.09	.05
Urine neutrophil gelatinase-associated lipocalin, ng/mL	15.69 ± 23.35	39.14 ± 33.98	5.66 ± 5.08	.001

Table 3. Correlation Between Urine Neutrophil Gelatinase-Associated Lipocalin and Other Parameters

	All Patients (n = 50)		Normoalbumiurics (n = 38)		Microalbuminurics (n = 12)	
Parameter	r	*P*	r	*P*	r	*P*
Age	0.20	.10	0.10	.70	0.20	.50
Duration of disease	0.40	.007	0.30	.09	0.30	.30
Body mass index	0.20	.20	0.03	.90	0.10	.70
Body mass index (standard deviation score)	0.10	.50	-0.10	.70	0.20	.50
Systolic blood pressure	0.10	.40	-0.03	.90	0.10	.90
Diastolic blood pressure	0.04	.80	-0.03	.90	-0.20	.60
Urine albumin-creatinine ratio	0.50	.001	-0.02	.90	0.60	.05
Glomerular filtration rate	-0.20	.20	-0.10	.50	-0.40	.20
Hemoglobin A1c	0.50	.001	0.40	.02	0.80	.001
Serum high-density lipoprotein cholesterol	0.30	.07	0.10	.70	0.70	.007
Serum low-density lipoprotein cholesterol	-0.10	.60	-0.01	.90	- 0.40	.30
Serum total cholesterol	0.30	.03	0.40	.01	0.10	.70
Serum triglyceride	0.10	.10	0.10	.60	- 0.01	.90

and coworkers observed that the median of urine NGAL in microalbuminuric patients was higher than normoalbuminuric patients and controls.[13]

In our work, although the mean urine NGAL level was higher in normoalbuminuric patients compared to the controls, this difference was not significant; meanwhile, the microalbuminuric group showed significantly higher levels. Therefore, urine NGAL seems to increase in parallel with the severity of kidney disease, reaching higher levels in patients with manifestation of DN. Nielsen and colleagues reported that urine NGAL increased significantly with increasing albuminuria, as this tubular protein increased significantly from the normoalbuminuric to the microalbuminuric and further to the macroalbuminuric group, and they also reported that urine NGAL was higher in normoalbuminuric versus control patients.[14] In our study, urine NGAL positively correlated with albumin-creatinine ratio in microalbuminuric patients, denoting that the more severe the kidney affection, the higher the values of urine NGAL.

Interestingly, increased urine NGAL levels were found in 12 of 38 patients (31.6%) without early signs of glomerular damage, ie, in normoalbuminuric patients, that is to say tubular suffering as measured by urine NGAL might precede early signs of glomerular damage (microalbuminuria). These results are in accordance with recent studies that reported similar tendencies for urine NGAL in patients without appearance of pathological microalbuminuria, the early classic measurable sign of DN.[15] This attractive finding supports the growing hypothesis of a "tubular phase" of diabetic disease that precedes the manifestation of typical glomerular lesions. Thus, the increase in urine NGAL values may express the degree of subclinical tubular impairment, representing an earlier measurable index of renal distress compared with classic glomerular signs.[4,5,14,15]

After studying the level of serum and urine NGAL and their relations to albumin excretion rate in children with normal-range albuminuria (assumingly not having DN), Zachwieja and colleagues concluded that normal-range albuminuria did not exclude DN and that NGAL measurement could be more sensitive than microalbuminuria.[4]

On the other hand, 3 microalbuminuric patients in the present study had low urine NGAL (2.7 ng/mL, 7.6 ng/mL, and 4.2 ng/mL). It was not clear why these 3 patients showed low urine NGAL, but other factors than microalbuminuria alone were shown in our work to impact urine NGAL level, such as disease duration, hemoglobin A1c and glycemic control. These 3 patients had the least duration of the disease in the microalbuminuria group (6, 5, and 6 years, respectively) compared to much longer duration in other microalbuminuria patients. Moreover, they showed a rather good glycemic control (hemoglobin A1c, 7.5 mmol/mol, 7.4 mmol/mol, and 6.2 mmol/mol, respectively). Although ACEI were stopped adequately prior to sampling (1 week), we could not surely cancel any extended effect on urine NGAL as no prior basal levels of urine NGAL were obtained. Nevertheless, it is worth mentioning that these patients were on higher doses of ACEI prior to their stopping. The effect of using ACEI on decreasing urine NGAL was previously reported both in animal and human studies. Kuwabara and colleagues demonstrated that the angiotensin receptor blocker candesartan dramatically decreased urine NGAL excretion in lipoatrophic- and streptozotocin-induced mouse models of DN.[16] In addition, Nielsen and coworkers measured urine NGAL in a randomized cross-over study of 56 type 1 DM patients with DN treated with lisinopril 20 mg, 40 mg, and 60 mg daily. The study reported that urine NGAL was reduced by approximately 15% during 2 months of 20-mg lisinopril treatment, although the difference did not elicit statistical significance.[14]

Thrailkill and colleagues reported, as well as the current work, a positive correlation between urine NGAL and duration of the disease ($r = 0.25$, $P = .006$).[12] Other authors, however, reported the absence of this correlation in their results.[4,5] In the present study, a strong correlation is reported between values of urine NGAL and hemoglobin A1c as a measure of glycemic control of DM. The previous conclusion matches with the reports of the Diabetes Control and Complications Trial Research Group that has shown that there is a strong association between poor metabolic control and development of diabetic complications (DN included),[17] and also matches with the study of Rewers and colleagues, who recommended hemoglobin A1c target range for all groups of less than 7.5% and as teens approach adulthood, lower targets similar to those of adult population were suggested less than 7%.[18] This correlation was not

shown in other previous studies.[4,5,14,19]

Dyslipidemia is reported as a risk factor for the development of DN.[20] In the current study, we found a positive significant correlation between urine NGAL and cholesterol of all patients and a positive correlation between urine NGAL and cholesterol in normoalbuminuric patients, though some authors could not elicit this correlation.[5,19]

CONCLUSIONS

Urine NGAL had a positive correlation with albumin-creatinine ratio, duration of DM, hemoglobin A1c, and dyslipidemia. Also, positive urine NGAL results were found even in normoalbuminuric patients. We therefore can suggest that urine NGAL can be used as an early biomarker for DN in normoalbuminuric patients, especially those with long-standing DM, uncontrolled diabetes, and dyslipidemia; however, larger studies are still needed.

CONFLICT OF INTEREST

None declared.

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