Urinary Neutrophil Gelatinase-associated Lipocalin and Kidney Injury in Children With Focal Segmental Glomerulosclerosis

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Introduction. Neutrophil gelatinase-associated lipocalin (NGAL) is proposed as a marker of chronic kidney disease (CKD). This study was designed to find whether there is a correlation between urine NGAL and progression of kidney damage in children with focal segmental glomerulosclerosis (FSGS).
Materials and Methods. Data were collected at the initial diagnosis of FSGS and after 12 months of treatment based on the Mendoza protocol. Twelve children with FSGS and 15 healthy children were included. Urine NGAL was assessed at the initiation of the study in the two groups and after 1 year of receiving the treatment in the FSGS group.
Results. Urine NGAL was elevated in the FSGS group (350.0 ± 67.2 ng/mL) as compared to that in the control group (9.3 ± 3.8 ng/mL; $P < .001$), and there was a significant decline after 1 year (180.0 ± 45.9 ng/mL) in the FSGS group ($P < .001$). There were significant inverse correlations between urine NGAL and estimated creatinine clearance in the FSGS patients both at diagnosis (r = -0.589, $P = .03$), and after 1 year (r = -0.76, $P = .009$). There was a significant correlation between urine NGAL and urinary protein excretion in FSGS patients at diagnosis (r = 0.628, $P = .005$).
Conclusions. Urine NGAL in children with FSGS can be used as a marker of progression of kidney damage as expressed in its positive correlation with both declining in glomerular filtration rate and the level of proteinuria even in those with remission.

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INTRODUCTION

Focal segmental glomerulosclerosis (FSGS) refers to a set of particular histopathologic lesions in which steroid-resistant podocyte injury leads to patchy adhesions between the glomerular tuft and the Bowman capsule, followed by progressive glomerulosclerosis and proteinuric kidney failure.[1] Neutrophil gelatinase-associated lipocalin (NGAL) protein, also known as *lipocalin-2*, *siderocalin*, *uterocalin*, and *24p3*, belongs to the lipocalin family.[2] Neutrophil gelatinase-associated lipocalin is highly accumulated in the human kidney cortical tubules, blood, and urine, after nephrotoxic and ischaemic injuries.[3] It is significantly increased in patients with progressive but not stable kidney failure.[4] Urine and plasma NGAL concentrations increase proportionally to the severity and duration of kidney injury[5,6]; its concentration rapidly decreases with attenuation of kidney injury.[7]

Persistent proteinuria is an expression of kidney damage caused by numerous factors, but it also represents an important cause of kidney injury progression that can lead to chronic kidney disease (CKD).[8] In glomerular diseases, analysis

of isoenzymes of NGAL demonstrated that the increased urinary excretion of this enzyme is due to an increased release by the renal tubular cells and not to increased filtration across the damaged glomerular capillary wall.[9]

Serum creatinine requires several hours to days to accumulate, and therefore, it increases in serum only after 50% or more of kidney function is lost. Its concentration is also affected by multiple confounding factors.[10] From the former data, urine NGAL can be used as a marker of kidney injury in acute conditions; accordingly, this study hypothesized that urine NGAL is a marker of disease progression in FSGS children and it can represent the progression of disease in relation to their glomerular filtration rate (GFR). We tested this hypothesis by attempting to determine the urinary levels of NGAL in children with FSGS. Additionally, we investigated the relation between urine NGAL and progression of estimated creatinine clearance after 1 year of therapy for FSGS.

MATERIALS AND METHODS

Study Design

The medical record was reviewed to screen for preexisting kidney disease. Relevant demographic data, system information, routine laboratory results, and medication regimens of all participants at the time of the study visits were obtained. Urine NGAL, estimated creatinine clearance, serum albumin, and 24-hour urinary protein levels were assessed in all FSGS patients from a frozen urine sample at the initial presentation and after 12 months of starting their therapy.

Participants and Baseline Data

The study was carried out in the Nephrology Unit of Pediatrics and Medical Biochemistry Departments, Faculty of Medicine, Zagazig University. The study protocol was approved by the ethical committee of Faculty of Medicine, Zagazig University. Written informed consent was obtained from each participant.

Twenty-three patients were diagnosed with FSGS in the Nephrology Unit of Pediatrics Department in the period from March 2010 to October 2011; five of them were excluded from the study as they needed other types of immunosuppressant during the period of the study. Four of the patients had decreased creatinine clearance at the start of the study, 1 lost to follow up, and 1 died with intracranial hemorrhage due to severe hypertensive encephalopathy. Twelve patients (7 boys and 5 girls; mean age, 7.4 ± 0.4 years) with FSGS (proved by renal biopsy) were categorized as the FSGS group. They were presented with steroid-resistant nephrotic syndrome in the period from March 2010 to October 2011. All of them were on the Mendoza protocol[11] plus angiotensin receptor blocker with a dose of 0.5 mg/kg/d to 2 mg/kg/d. Nephrotic-range proteinuria was defined as increased urinary protein excretion to greater than 40 mg/h/m^2, and remission was defined as urinary protein excretion less than 4 mg/h/m^2.[12] Only patients with normal creatinine clearance at the start of the study were selected. Creatinine clearance was estimated using the Schwartz formula.

The exclusion criteria were hypertension, peripheral inflammation disease, overt urinary tract infection, and receiving glucocorticoids or nephrotoxic medications before the study period. All these conditions have been reported to raise urine NGAL level; recently, NGAL was identified as an early biomarker for acute kidney injury after cardiac surgery and as one of the most strikingly induced proteins in the kidney after ischemia. Thus, NGAL might represent an early, sensitive, noninvasive biomarker for acute kidney injury.[3,13-14]

Fifteen healthy children with comparable age and sex distributions with those of the FSGS group (9 boys and 6 girls; mean age, 8.0 ± 0.2 years) were recruited from the pediatrics outpatient clinics of Zagazig University Hospital. They were negative for microscopic hematuria and proteinuria.

Biochemical Analyses

Venous blood samples were drawn from all participants, and sera were separated immediately. Five milliliters of urine was obtained, spun at 3000 rpm for 15 minutes. The supernatant was aliquoted equally into cryovials and stored at -80°C until assay. Blood and urine samples were coded before assessment.

Serum albumin, serum creatinine, and urinary protein were measured by colorimetric methods (Spinreact, SA Ctra, Santa Coloma, Spain). The NGAL level in urine was assessed by an NGAL enzyme-linked immunosorbent assay kit (Kit 036, AntibodyShop, Grusbakken, Denmark), which specifically detects human NGAL. The subjects

were advised not to participate in vigorous physical activities 1 day before the examination. The assay was performed according to the manufacturer's protocol: 100 µL of NGAL standards or diluted samples of urine were applied to the precoated microwells in duplicates. Microwells were then incubated for 1 hour at room temperature and then washed with washing buffer. In succession, biotinylated NGAL antibody and horseradish peroxidase-streptavidin were incubated in the wells for 1 hour each with shaking. Tetramethylbenzidine dihydrochloride substrate was added for 10 minutes in the dark before adding stop solution. Finally, NGAL concentration was measured at 450 nm wavelength. The absorbance was proportional to the concentration of NGAL. A standard curve was constructed by plotting absorbance value versus NGAL concentration of standards, and concentrations of unknown samples are determined using this standard curve. The intra-assay coefficients of variation were 2.1% (range, 1.3% to 4.0%). The interassay variation was 9.1% (range, 6.8% to 18.1%). Urine NGAL excretion is presented as the amount of urine NGAL in nanograms per milliliter.

Urinary creatinine concentration was used to normalize the NGAL measurements. The urinary levels of creatinine were analyzed by the Jaffe method. The urine NGAL levels were expressed as urine NGAL-creatinine ratio (ng/mg of creatinine).

Data Analyses

All statistical tests were performed using the the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA). The 1-way analysis of variance was used to compare the urine NGAL levels, serum creatinine, estimated creatinine clearance, and urinary protein between the study groups. The Spearman rho correlation coefficient was used to assess the correlation between urine NGAL and urinary protein and estimated creatinine clearance. Values were expressed as mean ± standard deviation. A P value less than .05 was considered significant.

RESULTS

Baseline Data and Kidney Function

Data of the participants are presented in Table 1. There were no significant differences between the two groups regarding age, weight, or height. There was no significant difference in serum creatinine level between the FSGS patients and the controls, neither at the start of the study, nor after 1 year of therapy (Table 2). There was a significant higher level of urinary protein excretion in the FSGS group (91.7 ± 16.0 mg/h/m^2) at the start of the study compared to the values after 1 year (30.0 ± 5.0 mg/h/m^2) and those of the controls (2.2 ± 0.8 mg/h/m^2). There was no significant differences in creatinine clearance between the FSGS group (89.4 ± 4.4 mL/min/1.73 m^2) and the control group (95.7 ± 6.4 mL/min/1.73 m^2) at the start of the study. However, creatinine clearance decreased significantly after 1 year of therapy (81.0 ± 12.5 mL/min/1.73 m^2) in the FSGS group (Table 2).

Neutrophil Aelatinase-associated Lipocalin

As shown in Table 2 and Figure 1, there was a significant elevation of the urine NGAL level in patients with FSGS during activity (350.0 ± 67.2 ng/mL) compared to the controls (9.3 ± 3.8 ng/mL; P < .05). On the other hand, urine NGAL decreased after 1 year of treatment with the Mendoza protocol

Table 1. Baseline Data of Studied Patients*

Parameter	FSGS Group (n = 12)	Control Group (n =15)	*P*
Age, y	7.4 ± 0.4	8.0 ± 0.2	.09
Body weight, kg†	25.2 ± 0.5	2.2 ± 0.8	.35
Height, cm	120.0 ± 7.2	131.5 ± 4.7	.10

*FSGS indicates focal segmental glomerulosclerosis.
†Weight of the patients group was measured in remission.

Table 2. Laboratory Studies*

Data	FSGS Group		Control Group	F	P
	Study Initiation	After 1 Year			
Serum creatinine, mg/dL	0.7 ± 0.2 (0.4 to 1.1)	0.8 ± 0.4 (0.4 to 1.7)	0.6 ± 0.2 (0.3 to 1)	2.4	.10
Urinary protein excretion, mg/h/m^2	91.7 ± 16.0 (60 to 110)	30.0 ± 5.0 (19 to 38)	2.2 ± 0.8 (1 to 4)	317.7	< .001
Creatinine clearance, mL/min/1.73 m^2	89.4 ± 4.4 (84 to 101)	81.0 ± 12.5 (63 to 104)	95.7 ± 6.4 (86 to 107)	10.4	< .001
Urine NGAL, ng/mL	350 ± 67.2 (200 to 435)	42.0 ± 17.6 (9 to 60)	9.3 ± 3.8 (5 to 21)	300.6	< .001
Urine NGAL/creatinine, ng/mg	27.6 ± 6.0 (12 to 38)	11.0 ± 2.1 (6 to 17)	2.8 ± 0.4 (0.9 to 4.1)	209.9	< .001

*FSGS indicates focal segmental glomerulosclerosis and NGAL, neutrophil gelatinase-associated lipocalin.

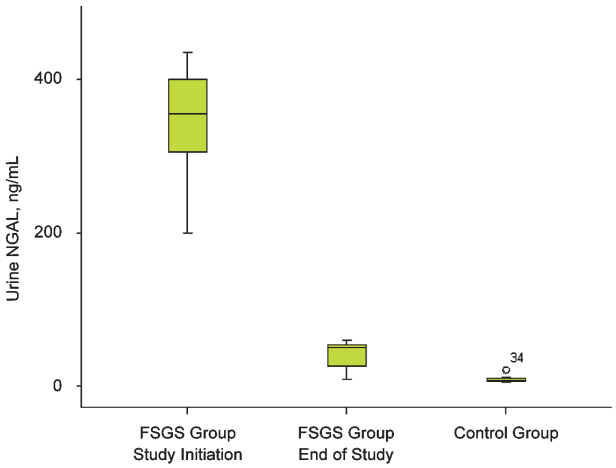

Figure 1. Urinary levels of neutrophil gelatinase-associated lipocalin (NGAL) in the studied groups.

(42.0 ± 17.6 ng/mL), but it was still significantly higher than that in the control group.

There was a significant inverse correlation between urine NGAL and creatinine clearance in the FSGS patients both in activity and after 1 year of treatment (Table 3). There was also a significant inverse correlation between urine NGAL-creatinine ratio and creatinine clearance in this group both in activity and after 1 year (Table 3). On the other hand, urine NGAL significantly correlated with urinary protein excretion in the FSGS patients in activity, but not at 1 year (Table 3). A same trend was observed for the relationship between urine NGAL-creatinine ratio and protein excretion.

There was no significant correlation between urine NGAL and serum creatinine (r = 0.075, $P > .05$; Figure 2).

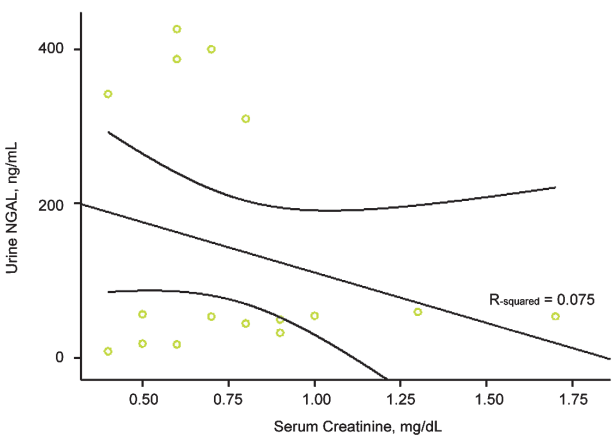

Figure 2. Correlation between urine neutrophil gelatinase-associated lipocalin (NGAL) and serum creatinine in the patients group.

Table 3. Correlations of Urine (Neutrophil Gelatinase-associated Lipocalin) NGAL and NGAL-Creatinine Ratio With Estimated Creatinine Clearance and Urinary Protein Excretion

	Creatinine Clearance		Urinary Protein Excretion	
Parameter	**r**	***P***	**r**	***P***
Urine NGAL				
Study initiation	-0.589	.03	0.628	.005
At 1 year	0.760	.009	0.431	.07
Urine NGAL/creatinine				
Study initiation	-0.613	.02	0.570	.009
At 1 year	0.691	.005	0.390	.09

DISCUSSION

Early identification of CKD and timely detection of progression are global priorities given the impending epidemic of CKD with which we are faced. It is now evident that even small deteriorations in kidney function, reflected by miniscule changes in serum creatinine or cystatin C levels, can have devastating consequences in patients with either acute kidney injury or CKD.[15-18] In addition to its enormous impact on survival and quality of life,[19] financial considerations in CKD have revealed that decreasing the decline in GFR by 10%, 20%, or 30% could result in cumulative health care savings over the next 10 years.

The present study clearly indicates that urine NGAL represents a novel risk marker of CKD progression. If predictive value of baseline estimated GFR confirms the general suggestion that an already impaired kidney function is an important factor for the subsequent progression of kidney damage, remarkably, urine NGAL showed an impressive predictive power in such a contest even after adjustment for GFR. This suggests that NGAL would not be a simple surrogate index of baseline estimated GFR, but a marker on its own, predicting CKD progression beyond the information provided by GFR estimation.[20]

In line with the present results, several studies have demonstrated that lipocalin level is significantly higher in patients with CKD than in healthy individuals. In the present study, significant elevation of the urine NGAL level in patients with FSGS during activity of the disease and after 1 year of treatment compared to controls. Furthermore, there was a significant elevation of the urine NGAL level in patients with FSGS during activity compared to its level after 1 year of treatment. This result was in agreement with

those of others who reported that NGAL might represent an early, sensitive, noninvasive biomarker for acute kidney injury.[21,22]

In this study, we demonstrated that no significant difference was present between estimated creatinine clearance in patients with FSGS during activity and the control group, while creatinine clearance decreased significantly after 1 year of therapy. On the contrary, there was no significant difference in serum creatinine level among the three groups. Furthermore, there was a significant negative correlation between urine NGAL and creatinine clearance in FSGS patients both in activity and after 1 year of treatment. Thus, as many studies declared that, NGAL could be suitable as an indicator of a reduced GFR even with a normal creatinine range.[22,23] Same authors added that decreased clearance played a role in CKD, leading to the accumulation of serum NGAL.[22,23]

Mori and Nakao[24] proposed an interesting theory which might explain the relationship between NGAL and GFR, suggesting that the increase in NGAL is not just the passive consequence of a reduced renal clearance. This hypothesis, called the "forest fire theory," assumes that the increase in NGAL in CKD ("forest fire") is the consequence of a sustained production by "inflamed" but vital tubular cells, whereas the rise in serum creatinine and the contraction of GFR are the mere passive result of a general loss of functional cells or nephrons. From this point of view, NGAL would represent a real-time indicator of how much active kidney damage exists within the overall condition of chronic kidney impairment.

The present study also revealed a significantly higher level of urinary protein excretion during the activity of FSGS, compared to its level in both after 1 year and the controls. In addition, there was a significant positive correlation between urine NGAL and urinary protein excretion in FSGS patients in activity, which was in agreement with Assal and colleagues.[22]

However, the present study has some limitations, as it was a single-center study, and the sample size may be considered relatively small, which restricts the power of conclusions that can be drawn. Therefore, further in-depth examinations should be undertaken to verify whether these findings can be confirmed in a longer observational period and to determine whether therapeutic measures targeting NGAL balance would be helpful in delaying the progression of CKD. Exclusion of children with CKD or receiving other immunosuppressants is a also a limitation that should also be acknowledged. In this study we highlightened this decrease in urine NGAL after 1 year of treating FSGS; however, the exact theory behind this decline either due to reduction of estimated GFR or administration of angiotensin-converting enzyme inhibitors needs further larger-scale studies with FSGS on Mendoza protocol alone versus those with Mendoza protocol and angiotensin-converting enzyme inhibitors.

CONCLUSIONS

We conclude that urine NGAL in children with FSGS can be used as a marker of progression of kidney damage as expressed in its positive correlation with both declining in estimated GFR and level of proteinuria even in those with remission.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Torban E, Bitzan M, Goodyer P. Recurrent focal segmental glomerulosclerosis: a discrete clinical entity. Int J Nephrol. 2012;2012:246128.
2. Flower DR. The lipocalin protein family: structure and function. Biochem J. 1996;318 (Pt 1):1-14.
3. Mori K, Lee HT, Rapoport D, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest. 2005;115:610-21.
4. Wasilewska A, Zoch-Zwierz W, Taranta-Janusz K, Michaluk-Skutnik J. Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of cyclosporine nephrotoxicity? Pediatr Nephrol. 2010;25:889-97.
5. McCormick T, Venn R. Recently published papers: Tracheostomy: why rather than when? Obesity: does it matter? And stroke: diagnosis, thrombosis and prognosis. Crit Care. 2007;11:127.
6. Bennett M, Dent CL, Ma Q, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol. 2008;3:665-73.
7. Haase M, Haase-Fielitz A, Bellomo R, et al. Sodium bicarbonate to prevent increases in serum creatinine after cardiac surgery: a pilot double-blind, randomized controlled trial. Crit Care Med. 2009;37:39-47.
8. Tryggvason K, Pettersson E. Causes and consequences of proteinuria: the kidney filtration barrier and progressive renal failure. J Intern Med. 2003;254:216-24.
9. Hultberg B, Ravnskov U. The excretion of N-acetyl-beta-glucosaminidase in glomerulonephritis. Clin Nephrol. 1981;15:33-8.

10. Herget-Rosenthal S, Marggraf G, Husing J, et al. Early detection of acute renal failure by serum cystatin C. Kidney Int. 2004;66:1115-22.
11. Tune BM, Mendoza SA. Treatment of the idiopathic nephrotic syndrome: regimens and outcomes in children and adults. J Am Soc Nephrol. 1997;8:824-32.
12. Clark AG, Barratt TM. Steroid-responsive nephrotic syndrome. In: Barratt TM, Avner ED, Harmon WE, editors. Pediatric nephrology. 4th ed. Philadelphia: Lippincott Williams & Wilkins;1999. p. 731-47.
13. Supavekin S, Zhang W, Kucherlapati R, Kaskel FJ, Moore LC, Devarajan P. Differential gene expression following early renal ischemia/reperfusion. Kidney Int. 2003;63:1714-24.
14. Devarajan P, Mishra J, Supavekin S, Patterson LT, Steven Potter S. Gene expression in early ischemic renal injury: clues towards pathogenesis, biomarker discovery, and novel therapeutics. Mol Genet Metab. 2003;80:365-76.
15. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol. 2005;16:3365-70.
16. Warnock DG. Towards a definition and classification of acute kidney injury. J Am Soc Nephrol. 2005;16:3149-50.
17. Fried LF, Katz R, Sarnak MJ, et al. Kidney function as a predictor of noncardiovascular mortality. J Am Soc Nephrol. 2005;16:3728-35.
18. Shlipak MG, Wassel Fyr CL, Chertow GM, et al. Cystatin C and mortality risk in the elderly: the health, aging, and body composition study. J Am Soc Nephrol. 2006;17:254-61.
19. Gorodetskaya I, Zenios S, McCulloch CE, et al. Health-related quality of life and estimates of utility in chronic kidney disease. Kidney Int. 2005;68:2801-8.
20. Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. Clin J Am Soc Nephrol. 2009;4:337-44.
21. Mishra J, Ma Q, Kelly C, et al. Kidney NGAL is a novel early marker of acute injury following transplantation. Pediatr Nephrol. 2006;21:856-63.
22. Assal SH, Elsherbiny A, Younes K, Radwan AS, Waked E. Assesment of serum neutrophil gelatinase-associated lipocalin versus protienuria in chronic kidney diseases. Kidney. 2008;17:289-93.
23. Mitsnefes MM, Kathman TS, Mishra J, et al. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. Pediatr Nephrol. 2007;22:101-8.
24. Mori K, Nakao K. Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. Kidney Int. 2007;71:967-70.

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