Original Article

Correlation between Urinary Neutrophil Gelatinase-Associated Lipocalin with Proteinuria and Glomerular Filtration Rate in Childhood Idiopathic Nephrotic Syndrome

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Abstract

Introduction: Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein belonging to the lipocalin superfamily, which is rapidly upregulated in response to renal injury and has proven to be an emerging biomarker for acute kidney injury (AKI). Recently it has been found that NGAL can also predict chronic kidney disease progression. Steroid resistant nephrotic syndrome has been found to be one of the important cause of chronic kidney disease in children. Therefore this study was conducted to evaluate whether urinary NGAL can correlate with proteinuria or glomerular filtration rate (GFR) in idiopathic nephrotic syndrome in children.

Objectives: To find out the correlation between urinary NGAL with proteinuria and GFR in childhood idiopathic nephrotic syndrome.

Materials and Methods: This cross-sectional was conducted in the Department of Pediatric Nephrology, Dhaka Shishu Hospital and Department of Pediatric Nephrology, Bangabandhu Sheikh Mujib Medical University from June 2014 to May 2015. Fifty-one children with idiopathic nephrotic syndrome were enrolled for the study. Urinary NGAL was measured using a commercially available HUMAN NGAL/LIPOCALIN 2 ELISA kit. The estimated GFR (eGFR) was calculated using revised Schwartz formula as follows: eGFR (ml/min/1.73m^2) = 0.413 x (height in centimeters/ serum creatinine in mg/dl). Correlation analysis of logarithmic value of uNGAL with logarithmic value urinary protein creatinine ratio and correlation analysis of logarithmic value of uNGAL with logarithmic value of estimated GFR were assessed by using Spearman rank correlation test.

Results: Among 51 children, 38 were in relapse and 13 were in remission. Urinary NGAL demonstated a significant ($p<0.001$) positive correlation with spot urine protein creatinine ratio. There was no significant correlation between urinary NGAL levels and GFR.

Conclusion: Urinary NGAL level significantly correlated with INS relapse but not with glomerular filtration rate.

Key-words: Urinary NGAL, Idiopathic nephrotic syndrome, Chronic kidney disease

Introduction

Human Neutrophil gelatinase-associated lipocalin (NGAL) was originally identified as a 25-kDa protein covalently bound to gelatinase from neutrophils[1]. It is expressed in neutrophil and in low levels in the kidney, prostate, and epithelia of the respiratory and alimentary tracts[2]. Neutrophil gelatinase–associated lipocalin is massively released from renal tubular cells after various injuring stimuli is emerging as a promising new biomarker for the early identification of acute kidney injury (AKI)[3]. Furthermore, any decrease in glomerular filtration rate would be expected to decrease the renal clearance of NGAL, with subsequent accumulation in the systemic circulation. Several studies reported that NGAL is emerging as a promising biomarker for the early detection and staging of CKD, for predicting progression, and for monitoring the response to interventions[4].

Clinical outcomes in nephrotic syndrome are determined by their histopathological diagnosis and responsiveness to steroid treatment. Steroid resistant nephrotic syndrome has been widely accepted as an increasing etiology of chronic kidney disease (CKD) in children[5]. Therefore, early detection of patients with increased risk of CKD by the identifications of urinary biomarkers has greatest significance. Plasma and urine NGAL in CKD were shown to correlate well with measured or estimated GFR[6]. Elevated levels of NGAL, as well as an inverse correlation with GFR, have now been documented in a number of patients with membranous nephropathy, primary focal segmental glomerulosclerosis, type-2 diabetic nephropathy and in a mixed population with CKD[7]. With this view, this study was carried out to evaluate the co-relation of urinary neutrophil gelatinase-associated lipocalin (uNGAL) with proteinuria level and GFR in children with INS for early prediction of clinical outcome.

Materials and Methods

This prospective cross-sectional study was carried out in the Department of Pediatric Nephrology, Dhaka Shishu Hospital and Department of Pediatric Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka from June 2014 to May 2015. Fifty-one biopsy proven children with idiopathic nephrotic syndrome between the ages of 1 and 18 years were selected for this study. Children below one year age and children with secondary nephrotic syndrome were excluded from the study. Informed consent was obtained from their legal guardians and ethical issues were approved by the local research ethics board. Demographic and clinical data were obtained at the time of enrollment and recorded in data collection sheet.

First-morning urine (about 2 ml) was collected from each patient for spot urinary protein creatinine ratio and urinary NGAL. Urine protein was measured by Pyrogallol red method by automated biochemistry analyzer (Siemens Xpand) and urinary creatinine was measured by Jaffe method by automated biochemistry analyzer (Siemens Xpand). Remission was defined clinically as bed side urine nil/trace on heat coagulation method for 3 consecutive days which was confirmed by spot urinary protein creatinine ratio of < 0.2 g/g. Relapse was defined as a measurement of ≥ 3+ on bed side urine heat coagulation test for at least 3 consecutive days, as confirmed by urine protein/creatinine ratio of >2 g/g. Urinary NGAL was measured using a commercially available HUMAN NGAL/LIPOCALIN 2 ELISA kit (Aviscera Biocience Inc, USA Lot no

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20111775) and all biochemical analyses were done in the Biochemistry Department of Dhaka Shishu Hospital. Urinary NGAL assay was done according to HUMAN NGAL/LIPOCALIN2 ELISA kit instruction (Code No SK00233-01, Formulation 96). Urinary NGAL assay employed the quantitative sandwich enzyme immunoassay technique. About 2 ml blood was collected from each patient and serum creatinine was measured by Jaffe method by automated biochemistry analyzer (Siemens Xpand). The estimated GFR (eGFR) was calculated using revised Schwartz[8] formula as follows: eGFR (ml/min/1.73 m^2) = 0.413 x (height in centimeters/ serum creatinine in mg/dl). Data were processed and analyzed using SPSS version 17.0 and expressed in medians with upper and lower quartiles for continuous parameters (IQR; 25^{th}; 75^{th} percentiles) and categorical variables were presented as frequency. For continuous variables, the difference was computed with Mann-Whitney U test and for categorical variables with Chi-squared test. Correlation analysis of logarithmic value of uNGAL with logarithmic value urinary protein creatinine ratio and correlation analysis of logarithmic value of uNGAL with logarithmic value of eGFR were assessed by using Spearman rank correlation test. Level of significance was set at 0.05 and p <0.05 was considered significant.

Results

Total 51 children with idiopathic nephrotic syndrome were enrolled in the study. Among them, 12 patients were MCD and 32 were MesPGN, FSGS and MPGN were 3 each and 1 was Membranous GN (Table-I). Table-II showed that there were no significant differences between the 2 groups regarding median age, weight, height and sex. Table III showed that median [IQR] uNGAL concentration was significantly (p=0.004) higher in patients with other than MCD Groups in comparison to patients with MCD (130 [85- 172] vs. 44.5 [32-109.5ng/ml, respectively), but no significant difference observed between children in relapse and remission group. Regarding spot urine protein creatinine ratio, median eGFR, s. albumin and s. creatinine no significant differences were noted between 2 groups. Figure-1 showed scatter diagram depicting positive correlation between uNGAL and urinary protein creatinine ratio in children with INS. The correlation coefficients using Spearman rank correlation was statistically significant (r= + 0.51; p<.001). Figure-2 showing scatter graph with no significant correlation between uNGAL and eGFR estimated with revised Schwartz formula. The Spearman correlation coefficient was (r= -0.181p>0.05) which was not statistically significant.

Table-I: Histological diagnosis of the patients

Histopathology Findings	Frequency	Percentage
MCD	12	23.5
MesPGN	32	62.7
FSGS	3	5.9
MPGN	3	5.9
MEMB	1	2.0
Total	51	100.0

Table-II: Descriptive demographics according to clinical parameters

Variables		Total (n=51)	MCD (n=12)	Other than MCD (n=39)	*p*-value	Children in relapse n=38	Children in remission n=13	*p*-value
		Median (IQR)	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Clinical parameters	**Median age** (months)	96 (60; 144)	90 (63; 153)	96 (43; 144)	0.938	10 (5.5; 13)	9 (4.7; 11.5)	0.32
	Median weight (kg)	26 [17; 38]	25(23.25;38.75)	26 [17; 37]	0.764	40.5(31; 53.2)	33.5(23.3; 59.2)	0.67
	Median height (cm)	124(100; 144)	124(100.75;141)	124 (96; 140)	0.876	147(134; 158)	138(120; 161)	0.42
	Gender: Male Female	28 23	6 5	22 18	--	--	--	--
	HTN	20	1	19	--	--	--	--
	Haematuria	8	0	8	--	--	--	--
	Clinical type SSINS SRINS	 41 10	 10 2	 31 8	0.769	--	--	--
Laboratory Parameters	**Median uNGAL** (ng/ml)	122(44-148)	44.5(32-109.5)	130(85-172)	0.004	4.6(2.1; 12.0)	3.5(1.7; 9.4)	0.68
	Spot urine protein creatinine ratio	5.54(3.5;8.47)	5.0(2.63; 6.8)	5.6(3.5;10.2)	0.272	6.8(4.5-9.4)	0.3(1.6-2)	0.32
	Median eGFR	96 (88; 03)	100(92.3;110.5)	94(86;101)	0.115	111(94; 129)	114(94;14)	0.69
	Median S. albumin (gm/L)	12.0(10.4-14.0)	10.8(10.0-12.0)	12.0(10.8-14.0)	0.86	--	--	--
	Median S. creatinine (mg/dL)	0.60 [0.48-0.80]	0.64(0.47-0.72)	0.59(0.48-.80)	0.92	--	--	--

Note: *p value calculated by Mann-Whitney U test.*

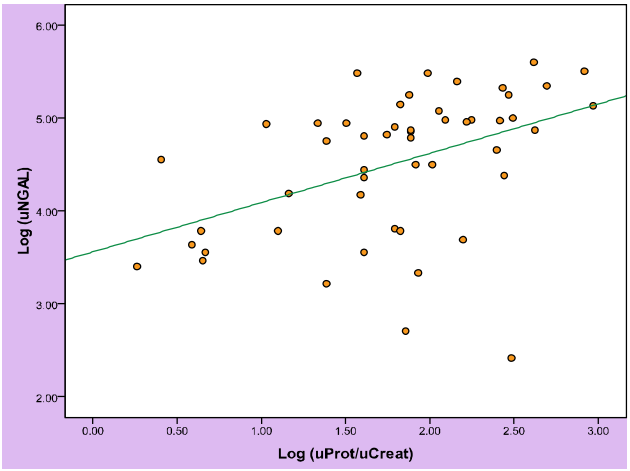

Figure-1: Relationship of logarithms of uNGAL with the logarithms of uProt to uCreat ratio.

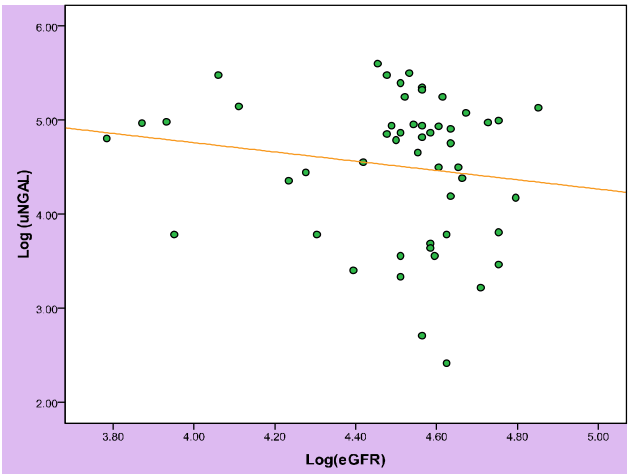

Figure-2: Relationship of the logarithms of uNGAL and eGFR

Discussion

This study was one of the few studies conducted to demonstrate the role of uNGAL in INS relapse and GFR. In this present study a significant positive correlation was observed between uNGAL and the proteinuria (p<0.001). Youssef DM and El-Shal AA[9] also found significant correlation between urine NGAL and urinary protein excretion in FSGS (r = 0.628, p = 0.005). Bolignano et al also found that uNGAL level were directly correlated with daily proteinuria level (*r* = 0.294, p = 0.01) in their study[10]. Bennett M R et al[11] conducted cross-sectional study among twenty-nine patients between the ages 2 and 19 years diagnosed with nephrotic syndrome (SRNS= 15 and SSNS=14) and found that median urine NGAL was significantly (p<0.001) higher in SRNS (172.3 ng/ml) than SSNS (6.3 ng/ml). They also found that NGAL levels demonstrated a significant negative correlation with glomerular filtration rate (r=0.5, p<0.001) and were independent of the degree of proteinuria in their study. The underlying pathophysiological mechanisms of uNGAL excretion in idiopathic nephrotic syndrome are still unknown. It can be explained by the fact that proteinuria decreases the absorption of low molecular weight tubular protein through competition for common transport mechanisms[12]. Under physiological conditions, serum NGAL is filtered through the glomerulus and almost totally reabsorbed by the proximal tubule through the multilig and protein transporter cubilin-megalin, which is particularly expressed in the brush border of tubular cells. In proteinuric diseases, an increased quantity of circulating NGAL could be lost through the damaged glomerulus as happens for other plasma proteins. Moreover, under these conditions, the megalin transporter becomes rapidly saturated by a massive protein overload, further decreasing the capacity of NGAL reabsorption. Megalin activity would be notably decreased as a consequence of proteinuria-induced damage of proximal tubular cells, especially in the brush-border area, where megalin mainly is located[13]. Indeed, several tubular proteins have been reported to be strictly involved in the experimental pathogenesis of tubular damage and its progression to terminal fibrosis and renal function detoriation[14].

This present study found no significant correlation between uNGAL and eGFR (p=0.20). Similarly, Chehade H et al[15] found no significant correlation between uNGAL and the uNGAL indexed to the uCreat and the eGFR (p= 0.26 and 0.45, respectively, with the revised Schwartz formula, and p = 0.57 and 0.48, respectively, with the new Quadratic formula).But Nishida et al [16] found contrasting results in their study, they measured serum and urinary NGAL levels in patients with several common pediatric renal diseases such as renal dysfunction (estimated glomerular filtration rate < 90 mL/min/1.73 m(2)) and found that both serum and urinary NGAL levels showed significant inverse correlations with an estimated glomerular filtration rate in the analysis with total subjects, and also in the analysis with the renal dysfunction group in urinary NGAL and the extent of proteinuria significantly correlated with urinary NGAL level. In a study conducted by Bolignano et al patients with membranous nephropathy and impaired renal function showed exaggeratedly increased baseline levels of neutrophil gelatinase-associated lipocalin (NGAL) and subjects with higher baseline NGAL showed considerably increased risk of worsening residual renal function within 1 yr compared with those with lower baseline NGAL values[17]. Mori and Nakao proposed a hypothesis ("forest fire") about the correlation between NGAL and glomerular filtration rate, suggesting that increased urinay NGAL is the consequence of a sustained production by "inflamed" but vital tubular cells, whereas increase in serum creatinine and glomerular function rate reduction are only due to loss of functional cells or nephrons[18]. There are a number of limitations to this study. First, the sample size was small. Secondly, no long-term follow up was given, therefore, whether higher initial NGAL levels predict worsening renal function cannot be determined from this study.

Conclusion

Urinary NGAL level significantly correlated with INS relapse but not with glomerular filtration rate.

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