Original Paper

Urinary Neutrophil Gelatinase-Associated Lipocalin and Its Association with the Histological Pattern in Idiopathic Nephrotic Syndrome

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Abstract

Introduction: Idiopathic nephrotic syndrome (INS) is the most common glomerular disorder of childhood. Clinical outcome of children with nephrotic syndrome depends on underlying histopathlogy and responsiveness to steroid treatment. Minimal change disease (MCD) has a favorable long-term prognosis whereas, other than minimal change nephrotic syndrome is often resistant to steroid and is more likely to progress to end-stage renal disease (ESRD). Neutrophil gelatinase-associated lipocalin (NGAL) which is a small protein belonging to the lipocalin superfamily has been demonstrated to be a powerful risk marker of chronic kidney disease progression. This study was undertaken to determine whether urinary NGAL could be used as a biomarker in differentiating minimal change disease from other glomerular histologic lesions in idiopathic nephrotic syndrome in children.

Objectives: To evaluate the association between urinary NGAL and histological pattern in idiopathic nephrotic syndrome.

Materials and Methods: This cross-sectional, multicenter study was conducted in the Department of Paediatric Nephrology, Dhaka Shishu Hospital and Department of Paediatric Nephrology, Bangabandhu Sheikh Mujib Medical University from June 2014 to May 2015. Fifty-one children with idiopathic nephrotic syndrome comprising 12 children with minimal change disease (MCD) and 39 with other than minimal change nephrotic syndrome were included. Urinary NGAL was measured using a commercially available HUMAN NGAL/ LIPOCALIN 2 ELISA kit which employed the quantitative sandwich enzyme immunoassay technique. Median urinary NGAL level were compared between MCD and other than MCD. Median urinary NGAL and urinary creatinine ratio also compared between two groups. The prognostic accuracy of urinary NGAL was assessed by receiver operating characteristic (ROC) curve analysis.

Results: Median urinary NGAL (uNGAL) level of MCD group was 44.5 [IQR: 32-109.5] (ng/ml) and that of the other than MCD group was 130 [IQR:85-172] (ng/ml). This difference was statistically significant (p=0.004). Median urine NGAL and urine creatinine ratio was significant between two groups (MCD=105.5 ng/mg and other than MCD=288 ng/mg, p-value was<0.001). The area under the curve (AUC) for the uNGAL as a biomarker to differentiate MCD from other than MCD was 0.78 [95% CI: 0.64-0.92] (p=0.004) and showed an optimized sensitivity of 0.82 and specificity of 0.75 with an optimal trade-off value of 72 ng/ml.

Conclusion: Urinary NGAL was found to be a reliable biomarker to differentiate the histological pattern in idiopathic nephrotic syndrome.

Key-words: Idiopathic nephrotic syndrome (INS), Neutrophil gelatinase-associated lipocalin (NGAL), Minimal change disease (MCD), End-stage renal disease (ESRD).

Introduction

Nephrotic syndrome is a common childhood kidney disease characterized by nephrotic range proteinuria, hypoalbuminaemia, oedema and hyperlipidaemia[1]. Histologically minimal change disease (MCD) is the commonest (76.4%), other histological patterns are mesangial proliferative glomerulo nephritis (MesPGN) (2.3%), membranoproliferative glomerulonephritis (MPGN) (7.5%), focal segmental glomerulosclerosis (FSGS) (6.9%) and membranous nephropathy (1.5%)[2]. Clinical outcome in nephrotic syndrome is determined by histopathological diagnosis and responsiveness to steroid treatment. Steroid-sensitive idiopathic nephrotic syndrome (SSINS) is mostly due to minimal change disease (MCD) in 80% cases and has a favourable long-term prognosis while patients with FSGS and those with diffuse mesangial proliferation are often resistant to corticosteroid treatment and progress more often

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to renal failure[3]. Recently few studies have suggested that urinary neutrophil gelatinase-associated lipocalin (uNGAL) can be used to distinguish steroid sensititive idiopathic nephrotic syndrome (SSINS) from steroid resistant idiopathic nephrotic syndrome (SRINS) and can also be used as a reliable biomarker for differentiating MCD and FSGS[4,5]. However, the data regarding the use of this novel biomarker in nephrotic syndrome is only limited to few studies and to date no studies evaluating the role of urinary neutrophil gelatinase-associated lipocalin (uNGAL) in a various histological pattern in idiopathic nephrotic syndrome have been assessed in Bangladesh. With this view, this study was done to evaluate the role of urinary neutrophil gelatinase-associated lipocalin (uNGAL) in differentiating minimal change disease from other than minimal change disease so that it may be used as a simple and noninvasive test for early prediction of clinical outcome in children with INS.

Materials and Methods

This prospective cross-sectional study was carried out in the Department of Paediatric Nephrology, Dhaka Shishu Hospital and Department of Paediatric Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka from June 2014 to May 2015. Fifty-one children with idiopathic NS between the ages of 1 and 18 years who underwent renal biopsy were enrolled in 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.

About 2 ml blood was collected in non-additive test tube 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 formula as follows: eGFR(ml/min/1.73 m2)= 0.413 x (height in centimeters/ serum creatinine in mg/dl). A percutaneous renal biopsy was done in all cases by Tru-Cut biopsy needle/ Bard Maxcore biopsy gun (14G/16G). All biopsy specimens were examined for light microscopic and direct immuno-fluorescence study in Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka.

First-morning urine (about 2 ml) was collected from each patient for spot urinary protein, urine creatinine 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). Urinary NGAL was measured using a commercially available Human NGAL/Lipocalin 2 ELISA kit (Aviscera Biocience) Inc, USA Lot no 20111775) and all biochemical analyses were done in the Biochemistry Department of Dhaka Shishu Hospital. Urinary NGAL assay was done according to Human NGAL/Lipocalin 2 ELISA kit instruction (Code No SK00233-01, Formulation 96T). A first-morning urine sample was collected and then centrifuged at 3000 rpm for 5 minutes, aliquoted and stored in a refrigerator at -20ºC until analysis. Urinary NGAL assay employed the quantitative sandwich enzyme immunoassay technique.

Data were processed and analyzed using SPSS (IBM SPSS Statistics for Windows, version 17.0, Armonk, NY, IBM Corp). Data were expressed in medians with upper and lower quartiles for continuous parameters (IQR; 25th; 75th percentiles) and categorical variables were presented as frequency. For continuous variables, the difference was computed with Mann-Whitney U test. Prognostic accuracy was assessed by receiver operating characteristics (ROC) curve analysis providing the area under the curve (AUC). 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 Minimal Change Disease (MCD) and 32 patients were Mesangial Proliferative Glomerulonephritis(MesPGN).The other notable findings were Focal segmental glomerulosclerosis (FSGS), Membranoproliferative glomerulonephritis MPGN (n=3 each) and Membranous GN (n=1) (Table-I).

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 showed that there were no significant differences between the 2 groups regarding median age, sex, weight and height. Regarding spot urine protein creatinine ratio, median eGFR, serum albumin and serum creatinine no significant differences were noted between the 2 groups (Table-III). The 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). Median uNGAL and median urinary creatinine ratio was also higher in other than MCD in comparison to MCD and the result was statistically significant (288 [176-328] vs. 105.5 [72-164.5] ng/mg, respectively; p<0.001) (Table-IV).

Table-II: Descriptive demographics according to clinical parameters

Variables		All patients (n=51)	MCD (n=12)	Other than MCD (n=39)	p-value*
		Median [IQR]	Median [IQR]	Median [IQR]	
Median age (months)		96 [60; 144]	90 [63; 153]	96 [43; 144]	0.938
Median weight (kg)		26 [17; 38]	25[23.25;38.75]	26 [17; 37]	0.764
Median height (cm)		124 [100; 144]	124[100.75;141]	124 [96; 140]	0.876
Gender	Male	28	6	22	-
	Female	23	5	18	-
HTN		20	1	19	-
Haematuria		8	0	8	-
Children on steroid		50	12	38	-
Children on cyclosporine & tacrolimus		6	1	5	-
Children on MMF		4	1	3	-
Children on cyclophosphamide		2	0	2	-

* p-value calculated by Mann-Whitney U test

Table-III: Descriptive demographics according to laboratory parameters

Variables	All patients (n=51)	MCD (n=12)	Other than MCD (n=39)	p-value*
	Median [IQR]	Median [IQR]	Median [IQR]	
Spot urine protein creatinine ratio	5.54 [3.50; 8.47]	5.0 [2.63; 6.8]	5.6 [3.5; 10.2]	0.272
Median eGFR	96 [88; 103]	100[92.3;110.5]	94 [86; 101]	0.115
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

* P value calculated by Mann-Whitney U test

Table-IV: Comparison of median uNGAL and median uNGAL / urinary Creatinine between MCD and other than MCD patients

Variables	All patients (n=51)	MCD (n=12)	Other than MCD (n=39)	p-value*
	Median [IQR]	Median [IQR]	Median [IQR]	
Median uNGAL (ng/ml)	122 [44-148]	44.5 [32-109.5]	130 [85- 172]	0.004
Median uNGAL/uCreatinine (ng/mg)	226 [114-312]	105.5[72-164.5]	288 [176-328]	<0.001

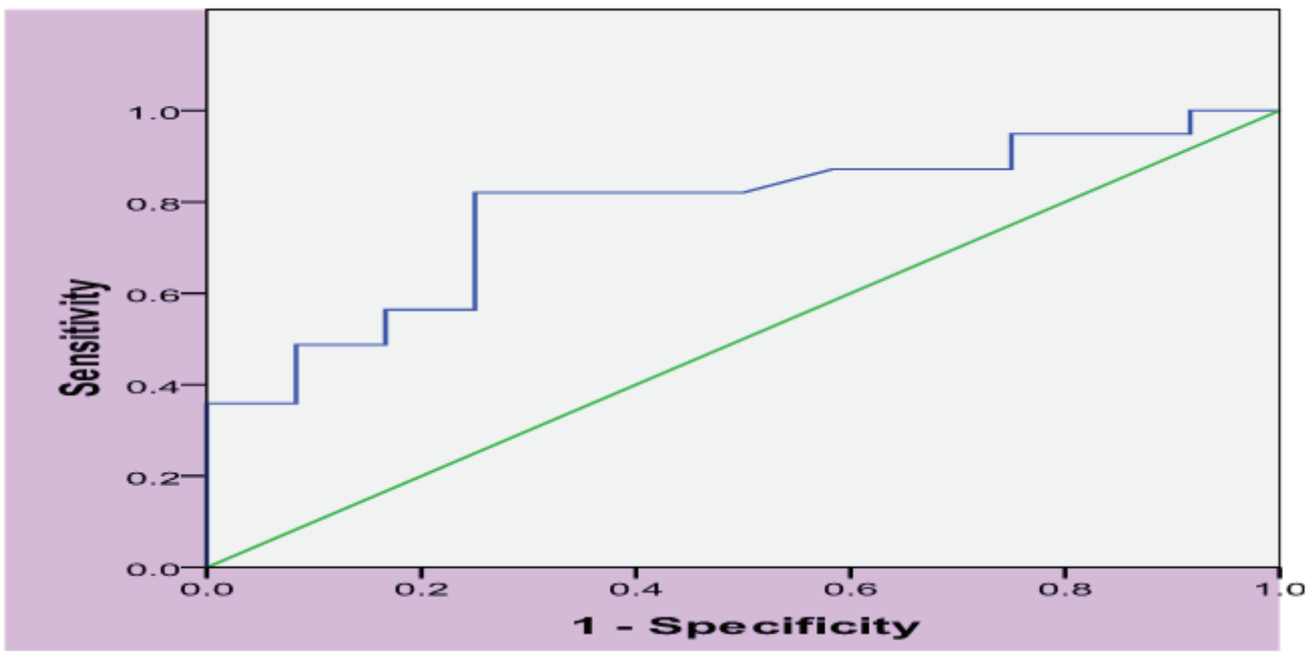

Fig-1: Receiver operator characteristic curves (ROC) for urinary neutrophil gelatinase-associated lipocalin (uNGAL) for differentiating minimal change disease from other than minimal change nephrotic syndrome.

Figure-1 shows receiver operator characteristic curves (ROC) for uNGAL. The area under the curve (AUC) for the uNGAL as a biomarker to differentiate MCD from other than MCD was 0.78 [95% Confidence Interval: 0.64-0.92] (p=0.004) which was statistically significant. Urine NGAL shows an optimized sensitivity of 0.82 and specificity of 0.75 with an optimal trade-off value of 72 ng/ml.

Discussion

The present study showed that median urinary NGAL level (uNGAL) was significantly different between MCD and other than MCD (44.5 ng/ml and 130 ng/ml respectively, p=0.004). Median uNGAL/u Creatinine was also found significantly different in both groups (MCD=105.5 ng/mg and other than MCD=288 ng/mg with p-value<0.001). This result was consistent with study conducted by Chehade H et al[5] in which median uNGAL level was significantly (p=0.01) higher in patients with FSGS than in patients with MCD (12.1µgm/L vs. 2.7 µgm/L, respectively) and the results remained unchanged when uNGAL was indexed to uCreat (8.3 vs. 1.0 µg/mmol, in FSGS and MCD group respectively; p=0.02).

Chehade H et al also showed that using a cut-off value of 17 ng/mg, the uNGAL/uCreat ratio enabled MCD to be differentiated from FSGS with an area under the curve (AUC) of 0.75 (p=0.01) and an optimized sensitivity of 0.77 and specificity of 0.78. In the present study the area under the curve (AUC) for the uNGAL as a biomarker to differentiate MCD from other than MCD was 0.78 [95% CI: 0.64-0.92] (p=0.004) and showed an optimized sensitivity of 0.82 and specificity of 0.75 with an optimal cut-off value of 72 ng/ml, thereby supporting the findings of Chehade H et al[5].

Bennett MR et al[4] 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). The area under the curve (AUC) for uNGAL to differentiate SRNS from SSNS was 0.91 (p<0.0001). But in their study, renal biopsy was done only in 14 out of 15 SRNS cases and FSGS was found in 13 and MCD was seen in 1 case. Among 14 SSNS group, only 1 patient had undergone renal biopsy which showed MCD. Therefore it cannot be concluded from their study that higher urinary NGAL level in SRNS reflect the difference between FSGS and MCD. Youssef DM and El-Shal AA[6] conducted prospective, longitudinal study among twelve children with FSGS and 15 healthy children. Data were collected at the initial diagnosis of FSGS and after 12 months of treatment based on the Mendoza protocol. They found that urine NGAL was elevated in the FSGS group (350.0±67.2 ng/mL) as compared to that in the control group (9.3±0.8 ng/mL; P<0.001) and there was a significant decline of urinary NGAL after 1 year treatment (180.0±45.9 ng/mL) in the FSGS group (P<0.001).

The underlying pathophysiological mechanisms of uNGAL excretion in idiopathic nephrotic syndrome are still unknown. Different hypotheses[7,8,9] have been postulated, such as injured glomerular barrier, abnormal cubulin-megalin transporter and secretion from injured tubular cells as an adaptive tubular response to injury). Mori and Nakao[10] proposed a hypothesis ("forest fire") 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 due to loss of functional cells or nephrons.

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. In this study, six patients were already on calcineurin inhibitor (CNI) during enrollment. Several studies[11,12] showed contrasting results regarding the impact of CNI on uNGAL excretion in children with INS. But the number of children included in this study was small and therefore, the impact of CNI on uNGAL excretion could not be evaluated.

Conclusion

The findings of present study denote that urinary NGAL can be used as a biomarker to differentiate the histological pattern in idiopathic nephrotic syndrome.

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