Original article

Child Kidney Dis 2019;23:105-110
DOI: https://doi.org/10.3339/jkspn.2019.23.2.105

ISSN 2384-0242 (print)
ISSN 2384-0250 (online)

The associations of Urinary Neutrophil Gelatinase-associated Lipocalin (NGAL) and Liver-type Fatty Acid-binding Protein (L-FABP) Levels with Hematuria in Children and Adolescents

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Received: 9 September 2019
Revised: 4 October 2019
Accepted: 10 October 2019

Purpose: We sought to determine associations of urinary neutrophil gelatinase-associated lipocalin (NGAL) and liver-type fatty acid-binding protein (L-FABP), known markers of renal injury, with hematuria in children and adolescents.
Methods: A total of 112 urine samples from 72 patients aged 2 to 18 years with hematuria were enrolled in this study. Urinary concentrations of NGAL and L-FABP were measured by ELISA and compared between subjects with and without proteinuria and between subjects with and without glomerulonephritis diagnosed by renal biopsy.
Results: Urinary concentrations of NGAL and L-FABP/creatinine (Cr) in subjects with proteinuria were not significantly different from those in subjects without proteinuria. They were not significant different between subjects with and without glomerulonephritis either. However, both concentrations of urinary NGAL and L-FABP/Cr were positively associated with urinary protein to creatinine ratio. Their levels had a tendency to be increased when proteinuria developed at later visits in subjects with hematuria only at initial visits.
Conclusion: Monitoring urinary NGAL and L-FABP levels in addition to conventional risk factors such as proteinuria and serum creatinine might improve the prediction of renal injury in pediatric patients with hematuria.

Key words: Neutrophil gelatinase-associated lipocalin, Liver-type fatty acid binding protein, Hematuria, Children

Introduction

Hematuria is a frequent abnormality in various renal diseases. Isolated hematuria without proteinuria has been considered as a benign condition. Proteinuria rather than hematuria is the main target for treatment and monitoring in renal diseases. However, epidemiological evidences have reported that persistent microscopic hematuria is a risk factor for progression to end-stage renal disease (ESRD)[1-3]. In a study including 1.2 million young Israeli adults, Vivante A. et al. reported that persistent asymptomatic isolated microscopic hematuria significantly increased the risk for developing ESRD (HR 18.5; 95% CI:12.4–27.6) after adjustment for age, sex, body mass index, and blood pressure[1]. In addition, in a large adult cohort enrolled 3,272 participants

with moderate chronic kidney disease (CKD), hematuria was found to be a significantly higher risk of CKD progression and death in the first 2 years of follow-up[2]. In a single-center cohort of 112 patients with IgA nephropathy followed for a mean of 14 years, Sevillano et al. reported that the proportion of patients reaching ESRD or a 50% reduction of renal function was significantly greater among patients with persistent hematuria than patients with minimal or negative hematuria, and disappearance of hematuria was associated with a lower decline in renal function[3]. Recently, several studies have suggested that hematuria might be a promoter of tubular injury and that tubular epithelial cells are key injury targets involved in hematuria-related renal damage[4-6]. However, little information has been obtained regarding the association between hematuria and tubular injury in children with hematuria. Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa protein of the lipocalin family[7]. It was originally identified in neutrophils, but is is also expressed in kidney in response to various pathologic states, such as inflammation, infection, ishchemia and neoplastic transformation[7,8] In normal condition, only low levels of NGAL are detectable in urine. However, following renal injury, NGAL is upregulated in the distal part of the nephron and this leads to increased urinary NGAL levels[9-11]. Liver-type fatty acid binding protein (L-FABP) is a protein expressed in the proximal tubule of the kidney[12]. In various stressful events causing renal tubulointerstitial injury such as renal ischemia, hyperglycemia and toxins, renal gene expression for FABP1 (fatty acid binding protein, liver) is upregulated and the urinary excretion of L-FABP is increased[13,14]. A number of studies have demonstrated that urinary NGAL and L-FABP might be noninvasive and suitable markers for renal tubular injury, not only for the early detection of acute kidney injury but also for the progression of chronic kidney disease[7,12,15]. Thus, the objective of this study was to examine the association between hematuria and tubular injury by evaluating two tubular injury markers, urinary NGAL and L-FABP, in children with hematuria.

Materials and methods

A total 72 children and adolescents aged 2 to 18 years who had persistent hematuria for more than 6 months and followed up at the Department of Pediatrics, the Catholic University of Korea, Bucheon St. Mary's Hospital between March 2016 and February 2018 were enrolled. Children who had transient hematuria coincided with such as urinary tract infection or fever, and had other chronic diseases such as cancers, diabetes mellitus and congenital anomalies of kidney and urinary tract (CAKUT) including cystic kidney diseases were excluded. After obtaining informed consent from subjects and their parents, medical histories including family history of renal diseases and anthropometric measurements including height and weight were collected from subjects during their visits to the clinic. Laboratory examinations including white blood cell counts, routine biochemical analysis, and urinalysis with microscopy were also performed. Hematuria was defined when ≥5 red blood cells were detected in a high-power field on microscopic analysis. Proteinuria was defined when a protein score ≥+1 (+1–+4) was obtained in urinalysis and random urine protein to creatinine ratio was ≥0.2 (mg/mg). Hypercalciuria was defined when the calcium to creatinine ratio in spot urine was ≥0.2 (mg/mg). Estimated glomerular filtration rate (eGFR) was calculated using a revision of the Schwartz equation: 0.413×height (in cm)/serum creatinine (mg/dl). The diagnosis of glomerulonephritis (GN) was made based on renal biopsy results except for post-streptococcal glomerulonephritis (PSGN). Renal biopsy was performed for children with hematuria if they had at least one of these criterion -proteinuria, decreased renal function, hypertension, decreased serum C3 level except for PSGN or parental anxiety. Total 30 patients were diagnosed with GN and their distributions were as follows- IgA nephropathy (n=19), IgA vasculitis with nephritis (n=5), Thin basement membrane nephropathy (n=2), mesangial proliferative GN (n=2), PSGN (n=1) and non-specific focal GN (n=1). Urine samples for measuring NGAL and L-FABP were obtained from children upon routine urine collection for urinalysis. Only one sample collection was performed for 46 subjects (46 samples) and more than two sample collections were performed for 26 subjects (66 samples). Therefore, 112 samples were collected. Fifty-four subjects (84 samples) had no proteinuria while 18 subjects (28 samples) had proteinuria. Forty-two subjects (54 samples) had not been diagnosed with GN while 30

subjects (58 samples) had been diagnosed with GN (Fig. 1). Of 26 subjects who offered more than two samples, 9 subjects had initial hematuria only and proteinuria during follow-up, 13 subjects had only hematuria, and 4 subjects had hematuria and proteinuria at every visit. Urine samples were centrifuged at 3000×g for 15 minutes at 4℃ within 30 min of collection and stored at -80℃ until final analyses. Urinary levels of NGAL and L-FABP were measured using ELISA kits for Human Lipocalin-2/NGAL Quantikine ELISA kit and Human L-FABP kits from R&D systems (Minneapolis, MN, USA), respectively, following the manufacturer's instructions. For detection of NGAL, urine was diluted 1:50. However, no dilution was required for the detection of L-FABP. Urinary levels of NGAL and L-FABP were normalized to urinary creatinine concentration (ng/Cr mg). All samples were run in duplicate. These levels were within the range of the standard curve.

Data are presented as mean±standard deviation for normally distributed values and median and interquartile range for nonparametric values. Categorical variables are expressed as frequencies and proportions. Differences between two groups were analyzed using unpaired Student's t-test (parametric distributions) or Mann–Whitney U test (nonparametric distributions). Associations between urinary NGAL or L-FABP levels and other clinical parameters were analyzed using Spearman's correlation coefficient r. All statistical analyses were performed using SPSS Statistics for Windows (version 20.0; IBM Corp., Armonk, NY, USA). *P* values <0.05 were considered significant.

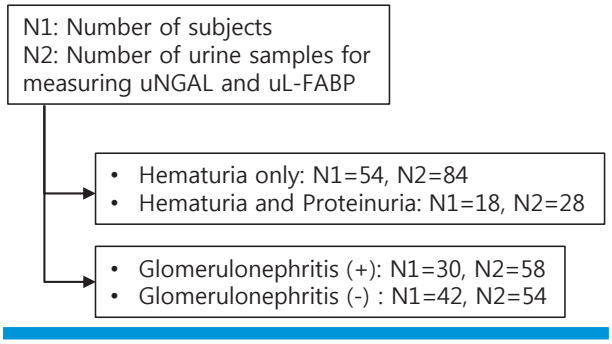

Fig. 1. Flow diagram to classify the participants and their urine samples according the presence of proteinuria and glomerulonephritis.

Results

1. Baseline characteristics and laboratory data of subjects with hematuria at initial visits

As shown in Table 1, sex distribution, hematuria duration and eGFR did not significantly differ between groups regarding the presence or absence of proteinuria or GN (i.e., with proteinuria vs. without proteinuria; with GN vs. without GN). However, subjects diagnosed with GN were older than subjects not diagnosed with GN. Frequencies of family history of renal or urologic diseases were higher in the non-GN group than those in the GN group. Diseases of the families in the non-GN group almost all originated from non-glomerular diseases such as renal stone. Subjects with proteinuria or diagnosed with GN had lower serum albumin levels than subjects without proteinuria or GN. Subjects without GN had higher urine calcium to creatinine ratio than subjects with GN.

Table 1. Demographic Data of Subjects at Initial Visit

	Proteinuria (-)	Proteinuria (+)	*P*	GN (-)	GN (+)	*P*
N	54	18		42	30	
Sex, male (%)	26, 48.15%	12, 42.86%	0.787	20, 47.62%	14, 46.67%	0.937
Age (years)	11.73±4.02	13.26±4.72	0.23	10.84±3.95	13.90±4.01	0.003
Duration of hematuria (months)	27.24±20.49	20.54±17.74	0.142	25.51±20.70	25.64±17.80	0.788
Family history of renal or urologic diseases	24, 33.33%	5, 27.78%	0.215	22, 52.38%	7, 23.33%	0.014
eGFR (ml/1.73m^2)	108.48±19.83	103.89±23.83	0.536	108.94±18.48	105.08±26.91	0.293
Serum albumin (g/dL)	4.7(4.5, 4.8)	4.3 (4.0, 4.5)	<0.001	4.7 (4.5, 4.88)	4.4 (4.3, 4.7)	0.008
Random urine protein to creatinine ratio	0.12 (0.08, 0.15)	0.32 (0.25, 0.6)	<0.001	0.14 (0.09, 0.2)	0.19 (0.1, 0.32)	0.185
Random urine calcium to creatinine ratio	0.07 (0.03, 0.12)	0.04 (0.02, 0.12)	0.589	0.085 (0.04, 0.16)	0.03 (0.02, 0.09)	0.017

Abbreviations: eGFR, estimated glomerular filtration rate calculated using a revision of the Schwartz equation: 0.413×height (cm)/serum creatinine (mg/dL); GN, glomerulonephritis.

2. Urinary NGAL/Cr and L-FABP/Cr values of children with hematuria

Table 2 shows urinary NGAL/Cr (uNGAL/Cr) and uL-FABP/Cr levels according the presence of proteinuria and GN at initial visits. These levels did not differ whether subjects had proteinuria/GN or not. Table 3 shows differences between uNGAL/Cr and uL-FABP levels at first sampling and at last sampling in 22 subjects from whom urine samplings were performed more than twice. Group 1 (n=9) included subjects who initially had only hematuria but had both hematuria and proteinuria at last visits, while group 2 (n=13) included subjects who had only hematuria at initial and last visits. Urinay NGAL/Cr and L-FABP levels between two groups did not differ at both initial and last visits. However, those levels at last visits had a tendency to be increased than those at fist visits only in group 1 (1.56 for uNGAL/Cr and 0.96 for uL-FABP/Cr), while the levels at last visits had a tendency to be decreased than those at first visits in group 2 (-2.92 for uNGAL/Cr and -0.11 for uL-FABP/Cr). However, only the difference value of uNGAL/Cr levels were significantly different between group 1 and group 2 (P=0.007).

Table 4. Associations between Urinary NGAL/Cr or L-FABP/Cr levels and Other Clinical Parameters

	uNGAL/Cr	uL-FABP/Cr
	Spearman's r (*P*)	
Male sex	-0.33 (<0.001)	-0.18 (0.058)
Age	0.091 (0.338)	-0.15 (0.115)
Hematuria duration	0.035 (0.717)	-0.045 (0.639)
Positive Family history of renal or urologic diseases	0.135 (0.157)	0.183 (0.054)
Urinary protein to creatinine ratio	0.267 (0.004)	0.457 (<0.001)
eGFR	0.048 (0.619)	0.15 (0.115)
Serum albumin	-0.09 (0.345)	-0.085 (0.37)

Abbreviations: uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin/creatinine; uL-FABP/Cr, urinary liver type fatty acid binding protein/creatinine; eGFR, estimated glomerular filtration rate calculated using a revision of the Schwartz equation:0.413×height (cm)/serum creatinine (mg/dL).

Table 2. Urinary NGAL and L-FABP Values of Subjects at Initial Visits

	N	uNGAL/Cr (ng/mg)	*P*	uL-FBAP/Cr (ng/mg)	*P*
Hematuria	54	2.96 (1.20, 8.87)	0.492	2.97 (2.09, 4.66)	0.172
Hematuria with Proteinuria (PU)	18	2.86 (1.32, 15.73)		4.25 (2.39, 6.4)	
Glomerulonephritis, GN					
(-)	42	3.47 (1.38, 9.79)	0.461	3.41 (2.63, 5.09)	0.235
(+)	30	2.41 (0.99, 5.74)		2.71 (2.01, 4.46)	
1) GN (-) PU (-)	42	3.47 (1.38, 9.79)		3.42 (2.63, 5.09)	
2) GN (+) PU (-)	15	2.99 (0.95, 4.28)		2.6 (1.49, 3.77)	
3) GN (+) PU (+)	15	2.06 (1.23, 17.14)		3.32 (2.39, 4.61)	
		1) vs 2)	0.267	1) vs 2)	0.082
		1) vs 3)	0.931	1) vs 3)	0.986
		2) vs 3)	0.533	2) vs 3)	0.187

Abbreviations: uNGAL/Cr, urinary neutrophil gelatinase-associated lipocalin/creatinine; uL-FABP/Cr, urinary liver type fatty acid binding protein/creatinine.

Table 3. Urinary NGAL/Cr and L-FABP/Cr Levels between Subjects who had Only Hematuria and Subjects who had both Hematuria and Proteinuria at Last Visits

	Group 1* (n=9)	Group 2† (n=13)	*P*
uNGAL/Cr levels at initial visits	0.57 (0.43, 1.58)	4.7 (1.1, 8.61)	0.126
uNGAL/Cr levels at last visits	2.06 (1.0, 28.48)	1.44 (0.1, 2.46)	0.209
uL-FABP/Cr levels at initial visits	3.17 (2.02, 6.54)	3.26 (2.74, 4.83)	0.948
uL-FABP/Cr levels at last visits	4.3 (3.62, 9.83)	3 (2.35, 5.77)	0.209
Difference value of uNGAL/Cr levels‡	1.56 (0.16, 27.97)	-2.92 (-9.52, 0.1)	0.007
Difference value of uL-FABP/Cr levels§	0.96 (-3.82, 7.81)	-0.11 (-1.09, 0.26)	0.357

*Group 1: subjects who initially had only hematuria but had both hematuria and proteinuria at last visits.
†Group 2: those who had only hematuria (n=13) at initial and last visits.
‡Differences of urinary NGAL/Cr levels: (the value of urinary NGAL/Cr at the last measurement)-(the value of urinary NGAL/Cr at the initial measurement).
§Differences of urinary L-FABP/Cr levels: (the value of urinary L-FABP/Cr at the last measurement)- (the value of urinary L-FABP/Cr at the initial measurement).

3. Correlations between urinary levels and other clinical variables

As summarized in Table 4, levels of uNGAL/Cr and uL-FABP/Cr were positively associated with urinary protein to creatinine ratio. Levels of uNGAL/Cr were negatively associated with male sex. However, levels of uNGAL/Cr or uL-FABP/Cr showed no association with other clinical parameters, including age, hematuria duration, family history, eGFR, or serum albumin level.

Discussion

Hematuria may be considered as the first sign of many glomerular diseases. Recently, several studies have suggested that hematuria is the forgotten CKD factor and that tubular injury promoted by hematuria might be associated with the development or progression to CKD[4,5]. The present study evaluated the association of hematuria with tubular injury through two tubular injury markers, uNGAL and uL-FABP.

NGAL is a 25 kDa protein of the lipocalin family[7]. L-FABP is a protein expressed in the proximal tubule of the kidney. It is involved in fatty acid metabolism[12]. In patients with acute kidney injury, increased levels of urinary NGAL and L-FABP can detect renal injury more sensitively than the conventional diagnostic criterion (i.e., increase of serum creatinine and/or decrease of urine output[10,16,17]. Several studies have reported that urinary L-FABP levels may reflect renal injury in early stages of nephropathy in patients with diabetes, even in normoalbuminuric state[14,18]. Smith et al. have shown that urinary NGAL in addition to conventional established cardiovascular and renal risk factors may improve the prediction of disease progression in patients with non-proteinuric stages 3 and 4 CKD[11]. Under the assumption that patients with glomerulonephritis or decreased eGFR or proteinuria in children with hematuria, compared patients without GN nor proteinuria or higher eGFR may have more tubular injury, we compared urinary NGAL and L-FABP levels based on these parameters. However, we did not find any difference. The present study was also limited by the small number of subjects in a single center. It did not contain healthy controls. Furthermore, some data were cross-sectional. However, we found that urinary levels were positively associated with proteinuria and that the development of proteinuria for patients with hematuria caused increases in levels of uNGAL/Cr and L-FABP/Cr. These results may suggest that monitoring uNGAL and L-FABP levels in addition to conventional risk factors such as proteinuria and eGFR may improve the prediction of renal injury in patients with hematuria. To confirm the role of uNGAL and uL-FABP as a marker for tubular injury in children with hematuria, further studies including longitudinal observations in a larger population and investigations of other tubular injury markers such as beta-2-microglobulin or N-acetyl-β-D-glucosaminidase (NAG) are needed.

Hematuria without proteinuria is regarded as a benign condition. Thus, many nephrologists only monitor patients' serum creatinine and proteinuria without performing renal biopsy. However, recent studies have reported that isolated hematuria is associated with the progression to ESRD[1,5]. Therefore, there is a continuing need for identifying more specific markers of renal injury besides relying on proteinuria or eGFR in patients with hematuria. Toward this goal, results of the present study suggest that monitoring urinary NGAL and L-FABP levels may help identify renal injury as an adjuvant method to standard risk factors in children with hematuria in a non-invasive manner. Further studies are warranted to investigate the role of these markers in the development or progression of renal injury in order to elucidate their values as prognostic and therapeutic targets for children with hematuria.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowlegements

This study was supported by the Institute of Clinical Medicine Research of Bucheon St. Mary's Hospital, Research Fund BCMC15YH07.

Ethics statement

The present study was reviewed and approved by the Institutional Review Board of Bucheon St. Mary's Hospital (IRB No. HC16SISI0010).

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