



Urinary neutrophil gelatinase-associated lipocalin might be an associated marker for anticipating scar formation in children with vesicoureteral reflux

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ABSTRACT

Introduction: Vesicoureteral reflux (VUR) is considered as the most common urogenital abnormality occurring in children. There is no reliable and routine clinical test that is non-invasive and rapid for recognizing the renal scars from VUR.

Objectives: Urine neutrophil gelatinase-associated lipocalin (uNGAL) can be the best indicator for early diagnosis of scar formation in children with VUR.

Patients and Methods: Children with primary VUR admitted to Hamadan's Besat hospital from March to December 2020 were included in this cross-sectional study. A dimercaptosuccinic acid (DMSA) scan was employed to assess all subjects in order to diagnose scar formation at least 180 days after the last episode of urinary tract infection (UTI). Additionally, uNGAL and its ratio to urine creatinine (uCr) levels were measured.

Results: During the study, all 63 cases (male/female, 13/50) with VUR were included for further evaluation. The mean age of the patients was 59.1 ± 34.7 months (range 2 to 132 months). Twelve subjects suffered from unilateral VUR, while bilateral VUR inflicted 51. According to the disease severity, nine patients had mild, 35 had moderate, and 19 had a severe form of VUR. No significant difference was observed between patients with ($n = 31$) and without ($n = 32$) renal scars regarding mean levels of the uNGAL and uNGAL/uCR ratios ($P > 0.05$).

Conclusion: We found no significant difference between the groups with and without the renal scar in terms of biomarker levels.

Implication for health policy/practice/research/medical education:

Urine NGAL/urine creatinine could be a non-invasive diagnostic biomarker for renal scarring due to primary VUR and might be used instead of radiographic or isotopic examinations.

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Introduction

Vesicoureteral reflux (VUR) as the most common urogenital abnormality occurring in children, is characterized by the retrograde urine flow from the bladder into the ureter and kidney (1). There are two primary and

secondary categories of VUR; the primary one occurs due to the ureterovesical junction anatomic malfunction in 1-2 percent of children (1, 2). In the presence of VUR, the risk of urinary tract infection (UTI), nephropathies and end-stage renal diseases (ESRD) notably increase (3).

By affecting approximately three percent of girls and one percent of boys before puberty, UTI is a significant childhood problem that can increase the risk of renal scarring, hypertension and ESRD (4, 5). In children with VUR, the incomplete emptying of the ureter and bladder enhance the likelihood of the occurrence of UTI, which can provide the rationale for renal damage and scarring called “reflux nephropathy” (4).

Most of the children with ureterovesical junction malfunction are diagnosed through radiologic assessments that are conducted for UTI (4). Our understanding of the association between VUR and renal scarring can help the earlier diagnosis and correction of this disease, through which further damages could be prevented. Although the current gold standard for late renal damages and scars detection is Tc-99m dimer cap to dimercaptosuccinic acid (DMSA), it is invasive because of the radiation exposure needed within the procedure (5). There is no reliable and routine clinical test that is non-invasive and rapid for recognizing the renal scars from VUR.

Neutrophil gelatinase-associated lipocalin (NGAL), also known as LCN2, is a 21-kDa lipocalin superfamily protein. It was first found in neutrophils that is a significant part of the innate immune response to bacterial infections (6). NGAL is also expressed under various pathological conditions in hepatocytes and renal cells (7). As a small secreted polypeptide, it can be detected in urine due to renal epithelial cell damage but not in physiological conditions (8,9).

Objectives

Due to the increased level of NGAL in urine through renal cell injury, it is considered as a favorable biomarker for the early detection of scar formation in children with VUR. Therefore, the aim of the present study was to investigate the level of urine NGAL (uNGAL) in the urine of patients with childhood VUR and UTI and their risk of renal scar formation in order to identify the availability of using uNGAL as a prognostic biomarker for renal scar formation.

Patients and Methods

Study design

Children with primary VUR were assessed in this cross-sectional study. Bottom-up approach protocol was employed to diagnose them during March and December 2020 (nuclear voiding cystourethrogram [VCUG] was conducted on patients with recurrent febrile UTI) in the pediatric nephrology department of Besat university hospital. Eighty-five patients with VUR between two months to 12 years old who were referred to the pediatric nephrology center were assessed. The subjects suffered from VUR that was made clear through nuclear VCUG. The number of the excluded subjects was equal to 22 because they did not come back for the follow-up examinations. The number of final subjects for assessment

was 63. Patients were evaluated through ultrasonography to remove cases with obstructive diseases and secondary VUR. VCUG and intravenous pyelogram (IVP) were used to assess the subjects with abnormal ultrasonography. Subjects included in the study had routine ultrasonography or abnormal ultrasonography without obstruction according to VCUG and IVP results. The study exclusion criteria were congenital or acquired immunodeficiency, metabolic disorder and recent surgical manipulations of the kidneys, ureters and bladder. Regarding the return of radiotracer to the ureter during nuclear voiding cystourethrogram, two classes, including mild (grade1), moderate (grade 2 and 3), and severe (grade 4 and 5), were considered for urinary reflux. All subjects were analyzed using a DMSA scan to find scar formation at least 180 days after the final UTI episode.

Samples and laboratory assessments

To analyze NGAL and urine creatinine (uCr) levels, voided urine samples of the first morning were taken from all patients. Urine samples that had pyuria were removed from the analysis. Sterile polypropylene containers were conducted to take the samples. Centrifuge at 4000×g for 10 minutes was used for one-milliliter aliquots and until biomarkers assessment, the supernatant fractions were stored at -80°C. In addition, microscopic analysis was conducted to test the existence of leukocytes or blood in urine samples, and then samples considered positive for blood or leukocytes were excluded. The compensated Jaffe method for Cr was employed to assess levels of urine Cr (mg/dL) in samples of fresh urine (Roche Diagnostics, Mannheim, Germany). Quantitative enzyme-linked immunosorbent assay (ELISA) kits were applied to determine levels of urinary NGAL (ng/mL) (Human NGAL Lipocalin-2/NGAL ELISA kit; Bio Vendor Diagnostic, Brno, Czech Republic). The procedure was based on manufacturer's instructions. In addition, all samples were tested for uNGAL/uCR (ng/mg) ratio. Biomarkers' levels of urine were shown as uNGAL ratio to urinary Cr.

Data analysis

Independent samples *t* test was conducted for comparing quantitative variables between two groups. Additionally the logarithmic scale of the quantitative data was employed to perform the *t* test. To compare more than two groups, variance analysis (ANOVA) together with a post hoc Tukey test was applied. To discern healthy members of the control group from those in the patient group, analysis of receiver operating curve (ROC) and area under the curve (AUC) were used as indicators for the ability of uNGAL and uNGAL/uCR ratio. Moreover, to better compare the age/sex-adjusted uNGAL and uNGAL/Cr ratio, an artificial neural network (ANN) model was used. ANN can show the non-linear relationship between independent and output variables with regard to independent variables

collection (such as gender, age and uNGAL/Cr). SPSS version 20 was used to run the estimations and $P < 0.05$ was considered statistically significant.

Results

Eighty-five subjects with VUR between 60 days to 12 years old who were referred to the pediatric nephrology center were evaluated. The subjects suffered from VUR that was made clear through nuclear voiding cystourethrography. The number of excluded subjects was equal to 22 because they did not come back for the follow-up examinations. The number of final subjects for assessment was 63 (male/female, 13/50). The mean age of the subjects was 59.1 ± 34.7 months (2 to 132 months).

As shown in Table 1, twelve subjects had unilateral VUR, and 51 subjects had bilateral VUR regarding the bilateral or unilateral forms of VUR. In addition, regarding the disease severity, nine cases had mild, 35 had moderate, and 19 had severe VUR form. DMSA scan examination was conducted for 63 patients at a minimum of 180 days after the previous febrile UTI; among them, 31 (8 males and 23 females) had a renal scar and DMSA scans for 32 cases were normal. Of eight male patients who had a renal scar, four subjects had severe bilateral VUR, two patients had severe unilateral VUR and two subjects showed moderate unilateral VUR. Of 23 female subjects who had a renal scar, four had severe bilateral VUR, 15 had moderate bilateral VUR, two had moderate unilateral VUR, while two were treated through surgery. No significant difference was observed between the patients with ($n=31$) and without ($n=32$) renal scars with respect to the mean levels of uNGAL and uNGAL/uCR ratios ($P > 0.05$).

Figure 1 presents the sensitivity and specificity of using the uNGAL /uCR ratio to predict renal scarring through DMSA scan in children with recurrent UTI and VUR using receiver operating curves (ROC). As shown in Figure 1, the biomarker could not be a significant predictor ($P=0.78$).

Discussion

The bacterial infection occurring in children, UTI, is a common cause of renal scar formation (10). Different factors facilitate this process and increase the possibility

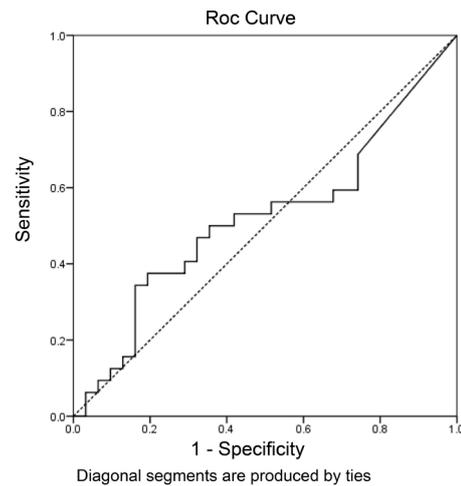


Figure 1. The sensitivity and specificity of using urine NGAL/urine creatinine ratio to predict renal scarring through DMSA scan in a child with recurrent UTI and VUR using receiver operating curves (ROC).

of renal scarring. Despite the age at presentation, gender, recurrent infection, peak fever, treatment delay, genetic susceptibility, and host defense factors, VUR is the most common congenital risk factor for UTI, leading to the scar of the kidney (reflux nephropathy) in 50% of pediatric cases (11-16). The pathological features of reflux nephropathy include interstitial fibrosis, cortical tissue loss and tubular destruction (16-19).

NGAL is a 21-kDa protein, may increase during renal inflammation. Ischemic renal tubules and damaged epithelium of the nephrons may also produce it (20). It is one of the latest indicators of renal injury. Unlike uCr levels and urine output measuring the renal function, damaged nephrons create NGAL and as a pathological biomarker, it releases into the blood and urine that is easily measurable (20). Several studies have shown that NGAL could be employed to diagnose acute kidney injury, tubular and intrinsic renal damage and identify the risk of developing the renal scars in children with UTI (21).

As a current method for diagnosis of renal scars, DMSA has at least 120 days delay, that makes the need for a more usable and accessible diagnostic method clear. The sensitivity and specificity of uNGAL for predicting renal scarring in patients suffering from VUR were evaluated

Table 1. The comparison of mean uNGAL and uNGAL /uCr between groups

Parameters	N	uNGAL			uNGAL/uCr			
		Mean (SD)	Median	P value	Mean (SD)	Median	P value	
Patients	63	7.1 (15.78)			0.20 (0.41)	0.042		
Severity	Mild	9	3.59 (6.64)	1.49	0.17	0.05 (0.08)	0.027	0.02
	Moderate	35	4.86 (7.18)	2		0.11 (0.17)	0.047	
	Sever	19	12.89 (26.7)	2.67		0.43 (0.66)	0.045	
Type	Unilateral	12	4.73 (8.52)	2.08	0.73	0.07 (0.11)	0.036	0.27
	Bilateral	51	7.66 (17.07)	2		0.23 (0.45)	0.047	

in this study.

Our results were inconsistent with the study of Parmaksiz et al, suggesting that uNGAL was a more sensitive biomarker than other factors for the prediction of renal scars in VUR (22). Alternately, Forster et al demonstrated that both uNGAL and plasma NGAL (PNGAL) could be used as novel sensitive markers for the early prediction of UTI (23).

Findings show that the assessment of both PNGAL and uNGAL in different disorders like UTI, VUR, renal scar and obstructive uropathy is greatly important for managing these kidney disorders (24-27). In the clinical setting, different implications have been assumed for these two types of NGAL, therefore PNGAL acts as an indicator for systemic inflammation, while uNGAL shows the injury of renal epithelium. No significant difference was found between the groups with and without renal scar in terms of biomarkers level. In addition, Nickolas et al argued that uNGAL did not increase in the mild cases of chronic kidney disease (28).

Conclusion

In conclusion, we found no significant difference between the groups with and without the renal scar in terms of NGAL/Cr level. As urine NGAL/Cr could be a non-invasive diagnostic biomarker for renal scar due to primary VUR and might be used instead of radiographic or isotopic examinations; further multi-center studies with large sample sizes are needed to confirm the potential application of serum and urine NGAL for both the diagnosis and the management of renal scars in children with VUR.

Limitations of the study

Due to the fact that this research was conducted in a clinic, our major limitations in conducting research are increasing the sample size and further multi-center studies in a larger population of patients are needed.

Authors' contribution

RA and JA were the principal investigators of the study. AH and RR were included in preparing the concept and design. FS and ZMA, GS, HB and JF revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the manuscript's content and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of

Helsinki. The Ethics Committee of Hamadan University of Medical Sciences approved the study protocol. The institutional ethical committee at Hamadan University of Medical Sciences approved all study protocols (#IR.UMSHA.REC.1399.143). Accordingly, written informed consent was obtained from all participants before any intervention. This study was extracted from a M.D., thesis at Hamadan University of Medical Sciences (Thesis #990223970). Besides, the authors have ultimately observed ethical issues (including plagiarism, data fabrication and double publication). The patient gave consent to publish as a case report.

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