

## Evaluating the Measurement of Urinary Neutrophil Gelatinase Associated Lipocalin for the Diagnosis of Vesicoureteral Reflux in Children

\*Alireza Eskandarifar<sup>1</sup>, Ramesh Rahehag<sup>2</sup>, Masoumeh Jafari<sup>3</sup>

<sup>1</sup> Assistant Professor, Department of Pediatric nephrology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.

<sup>2</sup> Assistant Professor, department of pathology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.

<sup>3</sup> Department of Pediatric, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.

### Abstract

**Background:** One of the most common urological disorders in childhood is vesicoureteral reflux (VUR) that can lead to kidney injury, scarring and chronic kidney disease. VUR is diagnosed by radiological methods, which are often invasive. We evaluated the possibility of diagnosing VUR by measuring urinary NGAL (Neutrophil gelatinase-Associated Lipocalin) in this study.

**Methods:** This prospective cohort study was conducted on children aged 1-5 years old with recurrent urinary tract infections in 2017- 2018 in Sanandaj, Iran. After ruling out other causes of recurrent urinary tract infections, urinary levels of NGAL and creatinine (Cr) were measured in random urine simultaneously with voiding cystourethrography (VCUG). Then the patients were divided into 2 groups with and without VUR based on VCUG and the collected data were statistically analysed.

**Results:** In this study, 71 children (12-60 months) with a mean age of  $23 \pm 36.35$  months were studied, among whom 34 children had VUR and 37 children did not have VUR. The mean urinary NGAL/Cr ratio in the group with VUR ( $3.45 \pm 3.8$  ng/mg) was significantly higher than that in the group without VUR ( $1.18 \pm 0.8$  ng/mg) (P: 0.002). The best cut-off point for urinary NGAL/Cr ratio was 1.27 ng/mg, with a sensitivity of 76.5% and specificity of 73% (ROC area: 0.76, P < 0.001).

**Conclusion:** Urinary NGAL/Cr ratio is higher in children with VUR and may be used as a rapid and non-invasive method in the diagnosis of VUR.

**Key Words:** Children, Diagnostic method, Neutrophil gelatinase-associated lipocalin, Vesicoureteral reflux.

\*Please cite this article as: Eskandarifar A, Rahehag R, Jafari M. Evaluating the Measurement of Urinary Neutrophil Gelatinase Associated Lipocalin for the Diagnosis of Vesicoureteral Reflux in Children. Int J Pediatr 2021; 9 (12):15015-15021. DOI: **10.22038/IJP.2021.57426.4508**

### \* Corresponding Author:

Alireza Eskandarifar, Assistant Professor, Department of Pediatric nephrology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran. Email: are1345@yahoo.com

Received date: Apr.30,2021; Accepted date:May.17,2021

## 1- INTRODUCTION

One of the most common urological disorders in childhood is vesicoureteral reflux (VUR). VUR refers to the return of urine from the bladder to the ureter and is caused by dysfunction of the ureter-bladder junction. High bladder pressure exacerbates VUR and increases the degree of reflux. VUR is usually asymptomatic and is often diagnosed during a urinary tract infection or neonatal hydronephrosis. VUR can lead to kidney injury and kidney scarring, which in the long run can cause hypertension, proteinuria and eventually chronic renal failure (1-6).

The diagnosis of VUR is based on radiological findings in the voiding cystourethrography (VCUG). This is an invasive method due to the need for bladder catheterization, which in addition to the risks of X-rays and psychological complications, sometimes leads to urethral damage and iatrogenic infections. So, most researchers intend to detect VUR as soon as possible by developing new non-invasive methods such as the use of novel urinary biomarkers like IL8, KIM-1 and NGAL (6 -10).

NGAL (Neutrophil gelatinase-associated lipocalin) belongs to the group of lipocalins. It is a small secretory protein weighing 25 kDa that is secreted by neutrophils and some epithelial and renal tubular cells. Its plasma level increases during inflammation or infection, especially in bacterial infections. Therefore, NGAL was initially considered as a marker of infection and some adenocarcinomas; then, its increase in urine was considered as a biomarker in acute kidney injury.

However, subsequent studies have shown an increase in urinary levels of NGAL in various pathological conditions such as urinary reflux, urinary tract infection and renal scar. The increase can be due to increased production or decreased

reabsorption of NGAL in the renal tubule (11-14).

Considering the frequency of urinary reflux in children and its role in the occurrence of urinary tract infections as well as its late complications, the importance of early diagnosis and the use of non-invasive diagnostic methods becomes apparent.

Therefore, we decided to evaluate urinary NGAL measurement as a rapid and non-invasive diagnostic method in urinary reflux by measuring and comparing urinary NGAL levels in children with and without VUR.

## 2- MATERIALS AND METHODS

### 2-1. Study design and population

This prospective cohort study was conducted on children aged 1-5 years old with recurrent urinary tract infections in 2017- 2018 in Sannandaj, Iran. A sample size of 70 was determined using the G Power software by considering a type 1 error of 0.05 and a statistical power of 0.95, equivalent to 35 people in each group. Sampling was performed by the availability method until the sample size was completed.

### 2-2. Method

Participants of this study included children aged 12 to 60 months who had referred to the pediatric nephrology clinic of Be'sat Hospital in Sanandaj, due to recurrent urinary tract infections. All patients were first thoroughly examined by a pediatric nephrologist. Renal tests including CBC, BUN, Cr, Na, K, VBG, Urinalysis and Ultrasonography were performed. In cases in which hydronephrosis or hydroureter was reported on ultrasonography, DTPA scans were performed to rule out obstructive disorders and stenosis. In the next stage, VCUG was performed 2 weeks after the complete cure of urinary tract infection and negative urine culture. DMSA scans were performed to rule out

renal scar in children with VUR. Demographic information including location, age, sex, weight, urinary creatinine, urinary NGAL and VCUG result were recorded in the designed questionnaire.

Patients were divided into two distinct groups according to the results of VCUG: 1. Patients with VUR 2. Patients without VUR.

Random urine samples were taken simultaneously with VCUG. The urine sample was kept at the appropriate temperature based on the conditions of the kit until reaching the required sample size.

### 2-3. Laboratory measurements

Urine NGAL was measured by ELISA method (Human Lipocalin2 / NGAL kit made by Boster, USA) and urine creatinine (Cr) was measured by Jaffe method. In order to eliminate the effect of urinary osmolarity on the results, NGAL/Cr ratio was calculated.

### 2-4. Ethical consideration

This study was conducted after the approval of the ethics committee of Kurdistan University of Medical Sciences (IR.MUK.REC.1393.173). The objectives of the study were explained to the parents of the children and after assuring them that the information was confidential, their informed consent to participate was obtained.

### 2-5. Inclusion and exclusion criteria

In this study, all children 12 to 60 months of age who referred to the pediatric nephrology clinic due to recurrent urinary tract infections were evaluated. Patients with underlying problems such as voiding disorders, anatomical abnormalities, urological disorders, renal dysfunction, renal scar, immunodeficiency and metabolic disorders, and chronic infectious diseases were excluded from the study

### 2-6. Data Analyses

The collected data were descriptively analyzed by SPSS software version 21 to determine the frequencies, means and standard deviations. Mean scores of the two groups were compared with independent t-test and Chi-square. Pearson correlation coefficient was used to determine the relationship between the two variables. Using receiver operating curve (ROC) and area under curve (AUC) and determining the best cut-off point, the specificity and sensitivity of urinary NGAL levels and urinary NGAL/Cr ratio in the diagnosis of VUR were calculated. Level of 0.05 was considered significant for all tests.

## 3- RESULTS

In this research, 71 children aged 12-60 months with a history of recurrent urinary tract infections were investigated. Their mean age of the participants was  $23 \pm 36.35$  months; 40 (56.34%) of them were girls and 31 (43.66%) were boys.

34 (47.89%) patients had VUR and 37 (52.11%) did not have VUR. In the group with VUR, 20 patients (58.82%), 10 patients (29.41%) and 3 patients (8.82%) and 1 patient (2.94%) had grade 1, 2, 3 and 4 VUR, respectively. In 4 patients (11.8%) there was bilateral VUR (**Table 1**).

The group with VUR included 13 (38.2%) boys and 21 (61.8%) girls and the group without VUR included 18 (48.6%) boys and 19 (51.4%) girls ( $P = 0.377$ ). The mean age in the group with VUR was  $19 \pm 1.14$  months and in the group without VUR was  $29.5 \pm 8.2$  months ( $P < 0.001$ ). The mean urinary NGAL level in the group with VUR was  $170.27 \pm 70.8$  ng/dl, and in the group without VUR was  $85.48 \pm 61.3$  ng/dl ( $P: 0.007$ ). The mean urinary NGAL/Cr ratio in the group with VUR was  $3.45 \pm 3.8$  ng/mg and in the group without VUR was  $1.18 \pm 0.8$  ng/mg ( $P: 0.002$ ) (**Table 2**).

In children with grade 1, 2, 3 VUR the mean urinary NGAL levels were 161±132 ng/dl, 171±139 ng/dl, 213±156 ng/dl, respectively; and the mean urinary NGAL/Cr ratios were 3.22±0.12 ng/mg, 3.55±0.5 ng/mg and 4.6±0.8 ng/mg, respectively, which showed a significant correlation between the grade of VUR and urinary NGAL levels ( $r: 0.386$ ,  $P: 0.001$ ) and urinary NGAL/Cr ratio ( $r: 0.391$ ,  $P: 0.001$ ).

ROC was used to determine the diagnostic value of urinary NGAL levels and urinary NGAL/Cr ratio in patients with VUR. Accordingly, the best cut-off point for urinary NGAL levels was 92 ng, at which the sensitivity was 85.3% and the specificity was 62.2% (ROC area: 0.73,  $P: 0.001$ ). For urinary NGAL / Cr ratio the best cut-off point was 1.27 ng / mg, and the sensitivity and specificity at this point were equal to 76.5% and 73%, respectively (ROC area: 0.76,  $P < 0.001$ ) (**Fig. 1**).

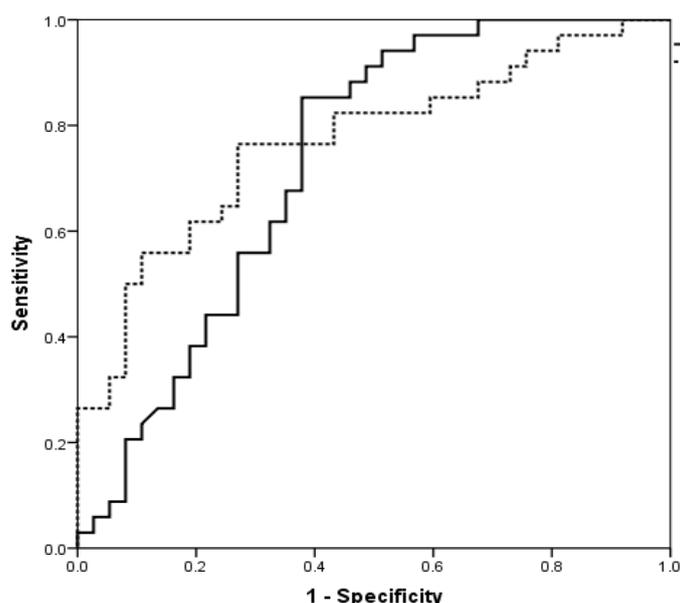
**Table-1:** Clinical and demographic features of all patients (n=71)

Quantitative variables	Mean ± SD
Age (months)	23 ± 36.35
Weight (kg)	15.49 ± 12.4
male	31 (43.66%)
female	40 (56.34%)
BUN (mg/dl)	12.57 ± 3.13
Cr (mg/dl)	0.55 ± 0.09
Na(meq/lit)	139 ± 3.6
K(meq/lit)	3.9 ± 0.5
Urine SG	1015 ± 8
Blood PH	7.39 ± 0.17
NGAL (ng/dl)	131.7 ± 87.3
NGAL/Cr (ng/mg)	2.36 ± 3.5
VUR -	37 (52.11)
VUR +	34 (47.89%)
VUR Grade 1	20 (58.82%)
VUR Grade 2	10 (29.41%)
VUR Grade 3	3 (8.82%)
VUR Grade 4	1 (2.94%)
Bilateral VUR	4 (11.8%)

**Table-2:** Comparison of different variable based on VUR

Variable	VUR -(group1)	VUR + (group2)	P-Value
Age	29.5±8.2	19±1.14	0.001
male	18(48.6%)	13(38.2%)	0.377
female	19(51.4%)	21(61.8%)	
NGAL (ng/dl)	85.48±61.3	170.27±70.8	0.007
NGAL/Cr (ng/mg)	1.18± 0.8	3.45±3.8	0.002

VUR: vesicoureteral reflux



**Fig. 1:** ROC curve based on the diagnostic value of the NGAL & NGAL/Cr ratios

#### 4- DISCUSSION

The goal of this research was to measure the urinary NGAL levels in children with VUR and compare it with children without VUR to evaluate its role as a urinary biomarker in the rapid and non-invasive diagnosis of VUR. The results revealed a significant difference between the urinary NGAL levels and urinary NGAL/Cr ratio in the two groups with and without VUR ( $P < 0.05$ ). ROC showed that the urinary NGAL/Cr ratio has a diagnostic value in VUR. The best cut-off point for urinary NGAL / Cr ratio was 1.27 ng / mg, and the sensitivity and specificity at this point were equal to 76.5% and 73%, respectively (ROC area: 0.76,  $P < 0.001$ ).

VUR is a known cause of neonatal hydronephrosis and recurrent urinary tract infections. Its prevalence is about 1% in the general population and 30-50% in recurrent urinary tract infections. 18% of children with chronic kidney disease had some degree of VUR (15). Currently, the gold standard for diagnosing urinary reflux is the use of radiology (VCUG), which in addition to being aggressive, has several

complications, so research on non-invasive methods with minimal complications can help in the rapid diagnosis of reflux.

In a study, Nikovar et al. (2020) examined and compared urinary NGAL levels and urinary NGAL/Cr ratio in two groups with and without primary VUR. The results showed that the mean urinary NGAL levels and urinary NGAL/Cr ratio in VUR group were significantly higher than the control group ( $P: 0.003$ ). With a cut-off point of 0.88 ng/mg, NGAL/Cr ratio, it had an acceptable diagnostic accuracy in the diagnosis of VUR, with a sensitivity of 84% and specificity of 81% (16). Their findings are almost consistent with our results in this research.

In another study on 50 infants with antenatal hydronephrosis, MohammadJafari et al. (2013) found that urinary NGAL/Cr ratios were higher in infants with VUR ( $P < 0.001$ ). In this study, the amount of NGAL increased with increasing grade of VUR (17).

Yet another study was conducted by Amiri et al., in 2020. They found a significant association between the grade of VUR and

urinary NGAL/Cr ratio ( $P < 0.05$ ) (18). Similarly, in our study, it was shown that urinary NGAL levels and urinary NGAL/Cr ratio increase along with the increase in the grade of VUR ( $P < 0.05$ ), which coordinates with increasing the risk of tissue inflammation and the risk of renal scar in the higher grade of VUR.

The finding of a study by Ichino et al. displayed an increase in urinary NGAL levels in patients with urinary reflux and Kidney parenchymal damage that was not related to the grade of VUR, so they suggested that NGAL be used to determine the necessity of surgery in these patients (19).

Another study was conducted by Parmaksız et al., in 2016. It demonstrated that the urinary NGAL/Cr ratio increases more in renal scars and has little correlation with the degree of VUR; and in patients with primary VUR, it is a sensitive and non-invasive diagnostic test to predict renal parenchymal damage (15). Given that in our study patients with renal scar were excluded from the study, we cannot comment on this.

In our study, children with VUR were younger ( $P < 0.05$ ), indicating that VUR is a self-limiting disease and decreases with age.

Additionally, although gender differences were not significant ( $P < 0.37$ ), the rate of VUR was higher in girls, which could lead to a higher prevalence of urinary tract infections and renal scarring among female children.

#### **4-1. Study limitation**

Significant limitations of our study were its conduct in one center and on a small number of patients; therefore, reconducting the research in a multi-center environment with a large sample size can help achieve more accurate results. Another limitation of our study was the performance of ultrasound and VCUG by

different radiologists, which is recommended to be performed by one radiologist, in future studies.

#### **5- CONCLUSION**

Based on the latest guidelines, VCUG should not be done routinely in febrile urinary tract infections, because it is an invasive method. This approach may increase the risk of undiagnosed VUR and its complications. Therefore, it is necessary to try to discover non-invasive methods. One of these methods is the use of novel urinary biomarkers. According to the results of this study and other studies, measurement of urinary NGAL/Cr ratio can help in rapid and non-invasive diagnosis of VUR before doing radiography. However, the role of other parameters such as age, gender and grade of VUR should not be forgotten.

#### **6- ACKNOWLEDGMENT**

We would like to thank the Kurdistan University of Medical Sciences and the Clinical Research Center of Be'sat Hospital (Sanandaj- Iran) for their cooperation and support.

**7- CONFLICT OF INTERESTS:** None.

#### **8- FUNDING/SUPPORT**

This study had no external funding.

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