IJKD KIDNEY DISEASES

Assessment of Urinary Level of Neutrophil Gelatinase-associated Lipocalin (NAGL) in Children with Renal Scar Due to Vesicoureteral Reflux

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Keywords. children, vesicoureteral reflux, renal scar, urinary neutrophil gelatinase-associated lipocalin (NGAL), diagnostic value

Introduction. Renal scarring is a serious complications of urinary tract infection and vesicoureteral reflux (VUR). The dimercaptosuccinic acid (DMSA) scan is the gold standard method for diagnosing renal scars but is an expensive procedure that risks ionizing materials and is not available to everyone. Neutrophil gelatinase-associated lipocalin (NGAL) increases following inflammation, infection, and acute kidney injury in the urine. The aim of this study was to evaluate the urinary level of NGAL and determine its diagnostic value in renal scarring.

Methods. Patients aged 3 to 60 months with pyelonephritis were included in this study. Voiding cystourethrography (VCUG) was performed in the presence of hydronephrosis on ultrasonography. Children with VUR underwent DMSA scans six months after successful treatment of pyelonephritis., Patients were divided into two groups based on the result of DMSA scan: those with and those without renal scars. Levels of urinary NGAL were measured in both groups.

Results. Ninety-two children with VUR (grades 2 to 5) were studied, of whom 40 had renal scars and 52 did not. The urinary level of NGAL at the cutoff point of 284 ng/dL had 70% sensitivity and 100% specificity for the detection of renal scars and was higher in patients with renal scars. ($P < .05$).

Conclusion. The urinary level of NGAL is considerably higher in children with renal scarring. It is not a good test for screening and early diagnosis due to its low sensitivity, although it can identify renal scars caused by VUR with high specificity.

IJKD 2023;17:14-9
www.ijkd.org
DOI: 10.52547/ijkd.6951

Original Paper

INTRODUCTION

A renal scar is formed when the functional renal parenchyma is replaced by fibrous tissue. Although the etiology of renal scarring is not fully understood at present, earlier studies have identified several risk factors for its formation, including old age, male gender, the severity of vesicoureteral reflux, and a history of urinary tract infection. Substantial renal scarring impairs kidney function and can lead to renin-mediated hypertension, chronic kidney disease, and preeclampsia in girls in the future.[1-6]

The diagnosis of renal involvement and cortical scar formation in cases of acute pyelonephritis is made by renal cortical scanning with Tc-99m dimercaptosuccinic acid (DMSA) scanning.[7-9] While a DMSA scan is highly sensitive to show

kidney inflammation and scarring, it is also very expensive, exposes children to ionizing radiation, and is not available in all medical centers. The use of urinary biomarkers is a relatively new method to assess renal function and renal injury in patients who experience episodes of pyelonephritis.[9,10]

Neutrophil Gelatinase-Associated Lipocalin is a 25-kDa protein that was first detected in neutrophils. It is produced at low, constant levels in a few other cells too, and plays a crucial role in the immune response to bacterial infections. In addition, NGAL is involved in several other pathways, such as innate immunity, programmed cell death, and epithelial cell growth and differentiation. NGAL can be measured naturally in healthy individuals with low levels in the blood and urine. However, more NGAL production has been reported in response to a variety of pathological conditions such as inflammation, infection, and acute damage to the kidney, liver, and epithelial cells.[11-15]

The urinary level of NGAL in acute kidney injury has recently attracted more attention than its serum level, which was initially thought to be a marker of infection and some adenocarcinomas. The urinary level of NGAL increases specifically with renal tubular cell damage, and earlier than elevation serum creatinine levels. NGAL is not only a rapid marker of acute kidney injury but is also linked to worsening clinical outcomes.[12,16,17]

Therefore, the study of the urinary level of NGAL has recently been considered by researchers for the rapid diagnosis of pyelonephritis and urinary reflux, and may also provide valuable information for the assessment of renal scarring in children. The measurement of NGAL is a simple, non-invasive, and low-cost method compared to a DMSA scan. So, we aimed to evaluate the urinary level of NGAL and its diagnostic value in children with renal scarring.

MATERIALS AND METHODS

This prospective cohort study was conducted between 2018 and 2020 after approval by the ethics committee of Kurdistan University of Medical Sciences (IR.MUK.REC.1395 / 165). The methods and goals were explained to the parents before the study, and their informed consent was obtained.

The sample size was determined to be approximately 95 people with the accuracy of 0.02 and reliability of 0.95, using the formula [n = (Z1 − / 2) 2 × P (1 −) / d2].

Patients aged 3 to 60 months who were referred to the pediatric nephrology clinic of Besat Hospital, Sanandaj, due to pyelonephritis were enrolled in our study. Initially, all children were thoroughly examined by a pediatric nephrologist. The diagnosis of a urinary tract infection was made based on clinical signs and symptoms, urinalysis, and urine culture.

Laboratory tests including the complete blood count (CBC), blood urea nitrogen (BUN), serum creatinine (sCr), sodium (Na), potassium (K), venous blood gas (VBG), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), and ultrasonography of the kidneys and urinary tract were performed. Voiding cystourethrography (VCUG) was done two weeks after successful treatment of pyelonephritis and obtaining a negative urine culture in patients with hydronephrosis on sonography. Children with vesicoureteral reflux (VUR) were included in the study, and those with known underlying problems such as anatomical abnormalities, urologic diseases, renal disorders, metabolic diseases, immunodeficiency syndromes, and other infectious diseases were excluded from the study.

In patients with VUR, a DMSA scan was performed six months later. Patients were classified into two groups: those with and without kidney scars, based on DMSA scan findings. Random urine samples were collected from all patients at the same time with the DMSA scan and stored in an appropriate environment according to the kit's instructions. The urinary level of NGAL was measured by the ELISA method (Human Lipocalin2 / NGAL kit, Boster, USA).

Statistical Analysis

The demographic data (sex, age, weight, urinary NGAL values, and DMSA reports) was entered into the designed questionnaire. These data were analyzed by SPSS software version 21 to describe the frequency, mean, and standard deviations. The means of the quantitative data in the two groups were compared by an independent t-test. The specificity and sensitivity of urinary levels of NGAL for the diagnosis of vesicoureteral reflux were calculated using the receiving-operational characteristic curves (ROC) and the area under the curves (AUC). A significance level of .05 was considered significant for all tests.

RESULTS

Ninety-two children with a history of febrile urinary tract infection and urinary reflux were included in this study. Patients included 33 boys (385%) and 59 girls (64.2%), who were in the age range of 3 to 60 months, with a mean age of 22.68 ± 13.97 months. Twenty-four patients had grade 1 reflux, 60 patients had grades 2, 3 and 4, and 8 patients had grade 5 reflux. Twenty-three patients (25%) had bilateral VUR. Forty children (43.5%) had kidney scars, and another 52 (56.5%) had no scars based on DMSA scan results (Table 1). There were 14 boys (15.2%) and 26 girls (28.2%) among those who had renal scars, while there were 21 boys (22.8%) and 31 girls (33.7%) among the group who did not have renal scars ($P < .05$). The mean age was 28 ± 14 months in the group without scars and 19 ± 8 months in the group with scars ($P < .001$).

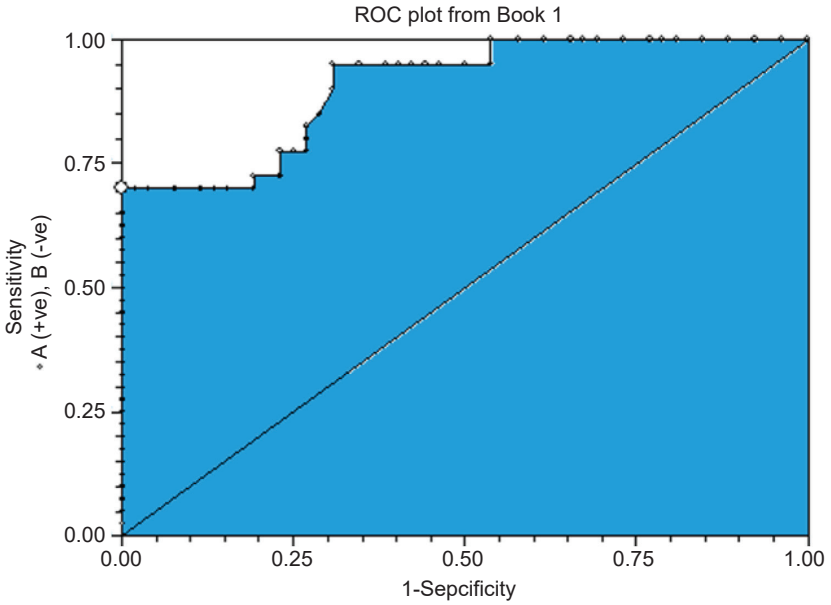
The mean urinary level of NGAL in the group of patients with renal scars was 524.05 ± 166.65 ng/dL and in the group without renal scars, was 125.77 ± 61.06 ng/dL ($P < .05$) (Table 1). The positive and negative predictive values of urinary NGAL for detection of renal scars were calculated as 100 and 81.25%, respectively. The transverse and longitudinal axes in the ROC diagram showed the values of specificity and sensitivity, respectively, and the area under the curve was 0.90, compared to the area under the curve for null hypothesis, which was 0.50, and significantly higher (Figure). As a result, there was a significant relationship between NGAL and renal scars ($P < .05$). It was showed that an NGAL level of 284 ng/dL was the optimal cutoff point, that is a level with a sensitivity of 70% and a specificity of 100%.

Clinical and Demographic Features of All Patients (n = 92)

Quantitative Variables	Mean ± SD
Age, mo	22.68 ± 13.97
Weight, kg	14.5 ± 13.6
Sex	
Male	33
Female	59
BUN, mg/dL	15.5 ± 4
Cr, mg/dL	0.7 ± 0.2
Na, meq/L	142 ± 3.2
K, meq/L	3.8 ± 0.6
Urine SG	1018 ± 5
Blood PH	7.42 ± 0.15
Patients With Scar	40
Patients Without Scar	52
VUR, Grade 1	24
VUR, Grade 2,3, and 4	60
VUR, Grade 5	8
Bilateral VUR	23
Urine NGAl Without Renal Scar, ng/dL	125.77 ± 61.06
Urine NGAl With Renal Scar, ng/dL	524.05 ± 166.65

DISCUSSION

VUR predisposes an individual to pyelonephritis by facilitating the transfer of bacteria from the



ROC Diagram of Specificity and Sensitivity of Values

bladder to the upper urinary tract. The inflammatory reaction can damage the kidney parenchyma and cause scarring, which is called reflux nephropathy. VUR-related scars may occur in early childhood in the absence of infection. The risk of developing VUR-related renal scars increase with higher degrees of VUR.[18]

The goal of our study was to assess the diagnostic value of urinary NGAL measurements in identifying renal scars in children with VUR. We demonstrated that the urinary level of NGAL had a positive predictive value of 100% and a negative predictive value of 81.25% and for prediction of renal scars, with a significantly higher levels in patients with renal scars. Also we could show the diagnostic value of the urinary level of NGAL in VUR, by the ROC curve and the sensitivity and specificity of urinary NGAL were 70 and 100, respectively, at the cutoff point of 284 ng/dL (ROC area: 0.9, $P < .05$).

NGAL can be found in both serum and urine, which have different origins and therefore can be used in different clinical contexts. In urinary tract infections, NGAL is secreted by neutrophils and increases in serum, indicating a systemic inflammatory response and pyelonephritis, whereas urinary NGAL is secreted by renal tubular epithelial cells, indicating kidney damage that could be caused by reflux, obstruction, or scarring. The use of urinary biomarkers for rapid diagnosis of urinary reflux and renal scarring has attracted more attention to this issue.[19-24]

A dramatic rise in NGAL was reported 2 weeks after pyelonephritis in a mouse model, which decreased within 4-6 weeks after recovery but never reached baseline. The initial increase appears to be due to an inflammatory response, but the persistently high amount after 6 weeks has a tubular origin, indicating tubular damage.[20,25,26]

The role of kidney injury molecule-1 (KIM-1), liver-type fatty acid-binding protein (L-FABP), and NGAL biomarkers in predicting renal scarring due to VUR was investigated by Parmaksız *et al.* in 2015. Sensitivity and specificity were reported to be 72% and 60%, at the cutoff point of 0.58 µg/g of Cr, respectively.[27] In our study, only urinary NGAL was assessed and the sensitivity was similar to the study by Parmaksız, while it had higher specificity in our study, which may be due to different study methods.

Ghasemi *et al.* examined the association between urinary levels of NGAL and parenchymal involvement of the kidney following acute pyelonephritis in 2016. In this study, the measured urinary level of NGAL had a sensitivity of 67.4% and a specificity of 97.7% for the diagnosis of parenchymal involvement. Additionally, a positive predictive value of 96.7% and a negative predictive value of 76.7% were reported, which is similar to the results of our study.[28]

In a study by Rafiei *et al.*, 54 children diagnosed with acute pyelonephritis were divided into two groups of acute pyelonephritis with and without renal scars. Urinary levels of NGAL were measured in both groups. Urine NGAL levels were higher in the group with acute pyelonephritis and renal scarring ($P < 0.05$). The conclusion was that the assessment of the urinary levels of NGAL may help to identify children with acute pyelonephritis, who are at high risk for developing renal scarring.[29]

NGAL is a known bacteriostatic agent that also prevents erythropoiesis and leads to anemia. A study by Lee *et al.* on children with pyelonephritis found that the presence of anemia and elevated urinary levels of NGAL during hospitalization (> 150 ng/mL) were independent risk factors for renal scarring. They also showed that the urinary levels of NGAL are higher in children with non-febrile urinary tract infections, pyelonephritis, and pyelonephritis with a renal scar, respectively.[30]

Urinary tract infections can cause irreversible fibrosis due to inflammation, ischemia, and interstitial damage. Serum creatinine, blood urea nitrogen, creatinine clearance, urine analysis, and radiological findings have been traditionally used as indicators of kidney injury. However, these markers lack the sensitivity or specificity needed to diagnose kidney damage and kidney scarring, while a high urinary level of NGAL has been associated with kidney injury and the risk of kidney scarring.

The main limitation of our study was that it was conducted in a single center with a small sample size; therefore, future multicenter studies with a larger sample size are required. It is also recommended that the serum NGAL/Cr ratio be measured in addition to urine and serum NGAL levels in future studies.

CONCLUSION

The findings of the current study showed that

patients with renal scars following VUR had higher levels of urinary NGAL. Urinary NGAL is not considered a good test for screening and early diagnosis of renal scars caused by VUR due to its low sensitivity, but it can be used as a good diagnostic marker to identify renal scars because it has a high specificity.

ACKNOWLEDGMENT

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

CONFLICT OF INTEREST

The authors of this article have no conflict of interest.

FUNDING/SUPPORT

This study had no external funding.

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Received August 2022
Revised October 2022
Accepted December 2022