Original Article
http://mjiri.iums.ac.ir
Medical Journal of the Islamic Republic of Iran (MJIRI)

Med J Islam Repub Iran. 2024 (27 May);38.59. https://doi.org/10.47176/mjiri.38.59

Assessment of Urinary and Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) Levels as Novel Predictors for Vesicoureteral Reflux Diagnosis in Children with Febrile Urinary Tract Infection

Sahar Sadr Moharerpour[1,2], Hasan Otukesh[3], Rozita Hosseini Shamsabadi[3], Hossein Ghorbani[4]*, Shahrbanoo Nakhaie[5], Farnoosh Seirafianpour[6], Parsa Panahi[6]

Received: 14 Apr 2022 Published: 27 May 2024

Abstract

Background: The invasive, expensive, and time-consuming nature of radiological examinations for vesicoureteral reflux (VUR) has compelled researchers to search for new markers to predict VUR. This study was designed to evaluate the usefulness of serum and urine concentrations of neutrophil gelatinase-associated lipocalin (NGAL) in predicting the existence of VUR.

Methods: This cross-sectional study involved all patients with a first febrile urinary tract infection (UTI) referred to Ali Asghar Children's Hospital. Each patient included in the study had clinical symptoms of pyelonephritis and a positive urine culture. The patients were divided into 2 groups: VUR and non-VUR. The serum and urinary NGAL levels were calculated in both groups. The receiver operating characteristic (ROC) curve was used to look for serum and urinary NGAL cut-points that differentiated the VUR group from the non-VUR group.

Results: Among the 40 children in the study, 23 belonged to the VUR group. The median age was 2.5 years (range, 0.3-8 years), and 35 patients were girls. ROC curve analysis showed that only the urinary NGAL level was significantly related to VUR. There was no association between serum NGAL levels and VUR. According to the ROC curve, a urinary NGAL level cut-off value of 15 ng/mL was likely to be diagnostic of VUR with 82.6% sensitivity and 58.8% specificity.

Conclusion: The urinary NGAL level, specifically with a cut-off value of 15 ng/mL, can indicate the existence of VUR in patients with UTI with near-acceptable levels of sensitivity and specificity.

Keywords: Children, Diagnosis, Febrile Urinary Tract Infection, Neutrophil Gelatinase-associated lipocalin, Vesicoureteral Reflux

Conflicts of Interest: *None declared*
Funding: *None*

Cite this article as: Sadr Moharerpour S, Otukesh H, Hosseini Shamsabadi R, Ghorbani H, Nakhaie S, Seirafianpour F, Panahi P. Assessment of Urinary and Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) Levels as Novel Predictors for Vesicoureteral Reflux Diagnosis in Children with Febrile Urinary Tract Infection. *Med J Islam Repub Iran.* 2024 (27 May);38:59. https://doi.org/10.47176/mjiri.38.59

Introduction

Urinary tract infection (UTI) is one of the most common causes of morbidity in childhood (1-3). In children, UTI may be a key sign of abnormality in urinary system functioning (2). The most common anomaly associated with UTI is known as vesicoureteral reflux (VUR)—that is, the backward flow of urine from the bladder into the kidneys (4). Diagnosed through radiological procedures, VUR can be divided into 5 categories according to severity (4, 5). Accurate diagnosis and treatment of VUR are critical because reflux is a risk factor for the incidence of recurrent

Corresponding author: *Dr Hossein Ghorbani, Hossein.ghorbani@mubabol.ac.ir*

1. Non-Communicable Pediatric Diseases Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
2. Iran University of Medical Sciences, Tehran, Iran
3. Department of Pediatric Nephrology, Aliasghar Hospital, Iran University of Medical Sciences, Tehran, Iran
4. Department of Pathology, Rohanee Hospital, Babol, Iran
5. Pediatric Gastrointestinal Department, Aliasghar Hospital, Iran University of Medical Sciences, Tehran, Iran
6. Student Research Committee, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

↑What is "already known" in this topic:
Neutrophil gelatinase-associated lipocalin (NGAL) is an iron transporter protein secreted from human neutrophils and excreted from the proximal and probably also the distal tubules into the urine. Recent research has demonstrated that urinary NGAL levels increase in kidney damage.

→What this article adds:
With near-acceptable sensitivity and specificity, urine NGAL levels, especially with a cut-off value of 15 ng/mL, can identify the presence of vesicoureteral reflux in patients with urinary tract infections.

[DOI: 10.47176/mjiri.38.59]

UTI and pyelonephritis (6, 7). High-grade reflux and recurrent UTI lead to hydronephrosis, damage to the papilla and renal medulla, and eventually, renal scar formation (6, 7). Currently, the diagnosis of VUR involves voiding cystourethrography (VCUG), which is a time-consuming, costly, and invasive procedure (5).

Neutrophil gelatinase-associated lipocalin (NGAL) is an iron transporter protein secreted from human neutrophils and excreted from the proximal and probably also the distal tubules into the urine (8, 9). This protein plays a protective role by chelating iron from damaged tubules, thus preventing free radical formation and cell death (9). Recent research has demonstrated that urinary NGAL levels increase kidney damage (8, 10). The increase is intensive, particularly in acute kidney injury triggered by nephrotoxins or ischemic processes (8-10). It has also been proven that NGAL gene expression increases in the course of tissue damage, mainly if caused by bacterial infections (9, 11). Accordingly, enhanced NGAL gene expression is regarded as part of the immune response in the host body, which in turn eliminates bacteria in the early stages of infection (12). A previous study found that serum NGAL levels are increased in patients with acute bacterial infections (13). Thus, UTI is known to be one of the most important causes of elevated NGAL levels (14-16).

As mentioned before, VUR in children can be associated with severe complications, hospitalization, as well as costs for the healthcare system. Imaging modalities' invasive, expensive, and time-consuming nature has compelled researchers to find a noninvasive method for diagnosing VUR. In this regard, recent studies are looking for new markers to diagnose VUR to assess the prognosis in affected children and accelerate early treatment (17, 18). NGAL is a new marker whose value for predicting UTI has been reported in previous work (14-16). Recent research has also examined the role of NGAL in predicting or diagnosing other complications, including VUR; however, few studies have been published (19-22). Therefore, the clinical usefulness of increased urinary and serum NGAL levels as a reliable diagnostic marker remains uncertain. This study aimed to investigate the role of serum and urinary NGAL levels in predicting the existence of VUR in children with febrile UTIs.

Methods

Study Design

This was a cross-sectional study conducted in Ali Asghar Children's Hospital between June 2019 and September 2019.

Inclusion Criteria

All children aged younger than 10 years who exhibited central fever exceeding 38 degrees Celsius, dysuria, and urinary frequency, and whose urine cultures indicated significant bacterial growth >100,000 colony-forming units were enrolled in the study.

Exclusion Criteria

Patients who did not have a positive urine culture or a central fever >38 degrees did not enter the study. Patients older than 10 years were also excluded.

Data Collection

The existence of VUR was determined by VCUG or radionuclide cystography for all the patients; VCUG was performed 48 to 72 hours after initiating antibiotic treatment, following negative urine culture results. Data—including age, sex, serum, and urinary levels of NGAL (ng/mL), pyuria, leukocytosis, and erythrocyte sedimentation rate (ESR)—were meticulously documented using a specially crafted data collection form. Urinary and serum NGAL concentrations were measured via enzyme-linked immunosorbent assay (ELISA) post-UTI diagnosis but before commencing antibiotic therapy. Without filtration, NGAL values were not normalized by creatinine due to its exclusive secretion from the proximal tubule. Urine samples were cooled on ice and transported to the laboratory within an hour. Pyuria was defined as ≥10 white blood cells (WBCs) per high-power field in mid-stream urine. Leukocytosis was determined by a total WBC count of 12,000/μL or higher. Positive ESR values were ≥6 for girls and ≥26 for boys. Urinalysis also included assessments of leukocyte esterase and urinary nitrite levels for all patients. Parents were briefed on the research objectives, and they incurred no costs associated with testing.

Statistical Analysis

The relationship between the incidence of VUR and each qualitative variable was analyzed with the chi-square test. Mean serum and urinary NGAL levels were compared with t tests. A receiver operating characteristic (ROC) curve was used to look for serum and urinary NGAL cut-points that differentiated the VUR group from the non-VUR group. All data were imported into SPSS Version 26 for descriptive analysis and analytic treatment. $P < 0.05$ was considered statistically significant, and values for quantitative variables are reported as the mean ± standard deviation.

Ethical Considerations

Informed consent was obtained from the parents or guardians of all participants. The medical research ethical committee of the Iran University of Medical Sciences confirmed this study (research number: IR.IUMS.REC.2712, reference no: 1495674).

Results

Among the cohort of 40 patients included in our study, 23 were categorized in the VUR group, while 17 belonged to the non-VUR group. The mean age was 3.3 ± 2.7 years in the VUR group and 1.3 ± 1.09 years in the non-VUR group. Predominantly, infection by Escherichia coli was identified in 36 cases (90%), followed by Klebsiella in 2 cases (5%), enterococci in 1 case (2.5%), and Pseudomonas in 1 case (2.5%). Pyuria was evident in 18 patients (45%), leukocytosis in 32 (80%), and positive ESR in 35 patients (87.5%). A detailed breakdown of the laboratory findings for each group is presented in Table 1.

Statistical analysis revealed a significant association only between sex and the incidence of VUR, with no notable relationships found for other qualitative variables. Notably,

[DOI: 10.47176/mjiri.38.59]

Table 1. Demographic and Clinical Characteristics of the 2 Groups and Their Relationship With the Incidence of Vesicoureteral Reflux (VUR)

Demographic variables	Status	VUR	Non-VUR	*P*-value
Sex	Male	0	5	0.009
	Female	23	12	
Pyuria	-	14	8	0.523
	+	9	9	
Leukocyte esterase	-	21	15	0.990
	+	2	2	
Nitrite	-	20	13	0.432
	+	3	4	
Leukocytosis	-	4	4	0.702
	+	19	13	
Erythrocyte sedimentation rate	-	1	4	0.144
	+	22	13	

there was no marked discrepancy in mean serum NGAL levels between patients in the VUR and non-VUR groups. However, a notable disparity existed in mean urinary NGAL levels between the 2 groups ($P < 0.001$, VUR group: 95.8 ± 9.5, non-VUR: 85.6 ± 1.36). Moreover, the mean urinary NGAL level was significantly higher in patients with positive ESR than those with negative ESR ($P = 0.001$). However, no significant differences were observed in mean urinary NGAL levels between patients with and without pyuria ($P = 0.169$) or between those without leukocytosis ($P = 0.061$). Furthermore, no significant variance was noted in serum NGAL levels between patients with or without conventional markers of UTI—including ESR ($P = 0.240$), pyuria ($P = 0.066$), or leukocytosis ($P = 0.990$).

The ROC curve derived from serum and urinary NGAL levels is depicted in Figure 1. The area under the curve was calculated as 0.522 (95% CI: 0.272-0.772) for serum NGAL and 0.705 (95% CI: 0.545-0.862) for urinary NGAL. A cutoff value for urinary NGAL of 15 ng/mL exhibited a sensitivity of 82.6% and a specificity of 58.8% for predicting the incidence of VUR.

Discussion

The present study was designed to investigate the role of serum and urinary NGAL concentration in predicting the incidence of VUR. The ROC analysis showed no significant relationship between serum NGAL levels and VUR. Serum NGAL concentration did not yield acceptable levels of sensitivity and specificity for predicting VUR. In other words, no specific cut-off value for serum NGAL could be used to predict VUR unless it is used in conjunction with other diagnostic tools such as VCUG. However, urinary levels of NGAL can potentially predict the incidence of VUR with reasonable accuracy. A 15 ng/mL cut-off value for urinary NGAL level showed appropriate sensitivity (82.6%) and specificity (58.8%) for predicting VUR. Although these values cannot be regarded as definitive VUR predictors, they can facilitate prediction.

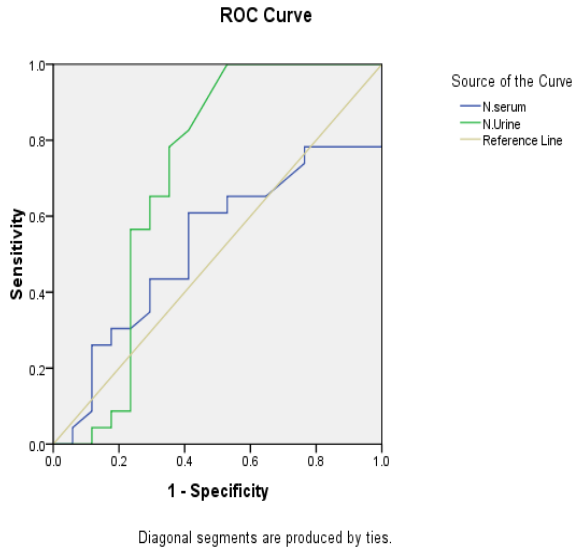

Figure 1. ROC curve for the association between serum and urinary NGAL levels.

Recent studies have investigated the role of new biomarkers, including NGAL, in predicting urinary system complications as an alternative to time-consuming and invasive procedures. In 2009, Yilmaz et al (23) argued for the first time that there is a relationship between urinary NGAL and the incidence of UTI in humans, and they were the first to demonstrate that urinary NGAL levels tended to be higher in children with UTI than healthy children. They concluded that a urinary NGAL concentration of 20 ng/mL can predict the existence of UTI with 97% sensitivity and 76% specificity. The participants in their research were children without acute kidney injury or chronic disease, whereas, in the present study, we suggest that NGAL can be used to predict VUR in patients with UTI; this may explain why our cut-off value differed from that obtained by Yilmaz et al. In accordance with their results, urinary NGAL levels in children with positive ESR were higher than in children with negative ESR in the present study. Other studies have reported that urinary NGAL is higher in patients with UTI than in healthy participants; these studies also concluded that urinary NGAL is a potentially good predictor of a diagnosis of bacterial infection or UTI (24-26).

In addition to investigating the role of NGAL in predicting UTI, some studies have focused on the role of NGAL in other urinary system complications. Seo et al (13) examined the role of NGAL in diagnosing pyelonephritis in infants with acute febrile UTI; they found that a plasma NGAL concentration of 61 ng/mL was predictive of acute pyelonephritis with 75% sensitivity and 78.3% specificity. In another study by Ichino et al (20), they demonstrated that NGAL could predict renal scarring in patients with VUR.

[DOI: 10.47176/mjiri.38.59]

In a similar study, Parmaksiz et al (19) examined the role of new biomarkers—for instance, NGAL, kidney injury molecule-1 (KIM-1), and liver-type fatty-acid-binding protein (L-FABP)—in the diagnosis of scarring in patients with VUR. Their results were consistent with those of Ichino et al, and they suggested that urinary NGAL was helpful in predicting renal scarring in patients with VUR. These earlier studies focused on complications in the urinary system or specific complications such as renal scarring.

Finally, only a few studies have specifically focused on predicting VUR using NGAL. Nickavar et al (27) conducted a prospective case-control investigation involving 69 young children, wherein they identified heightened urinary NGAL levels in patients afflicted with primary VUR. Notably, their diagnostic approach differed from the present study, as they relied on the urinary NGAL/urinary creatinine ratio for VUR diagnosis, revealing commendable specificity, sensitivity, and accuracy. Similarly, Amiri et al (28) conducted a study that yielded results that mirror the present findings. They, too observed elevated urinary NGAL levels in VUR patients. However, similar to the Nickavar study, they underscored the diagnostic efficacy of the urinary NGAL/urinary creatinine ratio for VUR detection. Nevertheless, despite the existing literature spotlighting the diagnostic utility of the urinary NGAL/urinary creatinine ratio for VUR diagnosis, the present study stands out as the pioneering endeavor in reporting diagnostic values solely based on urinary NGAL concentration. This emphasis on NGAL concentration as an independent diagnostic parameter signifies a notable advancement in the field, potentially offering a more streamlined and precise diagnostic approach for VUR.

The present study possesses noteworthy limitations that warrant acknowledgment. Primarily, the small sample size stands out as a critical drawback. Moreover, the omission of VUR grade consideration and its correlation with NGAL levels represents another limitation. These constraints underscore the need for future research to address these aspects comprehensively. Incorporating larger patient cohorts in subsequent studies would enhance the reliability of NGAL measurements and facilitate a more robust evaluation of its predictive capability, particularly among patients with advanced VUR grades.

Conclusion

The present analysis shows that serum NGAL levels were not useful in predicting VUR. However, urinary NGAL levels with a 15 ng/mL cut-off value may be a good predictor of VUR in patients with UTI. These laboratory findings in febrile patients with UTI can broaden our horizons regarding pediatrics and nephrology.

Authors' Contributions

H.G., S.S., and H.O.: study design and data analysis; S.S., R.H., and S.N: supervision and data gathering; all authors: writing and manuscript revision.

Ethical Considerations

The medical research ethical committee of Iran University of Medical Sciences confirmed this study (research No.: IR.IUMS.REC.2712, reference no: 1495674).

Acknowledgment

The authors thank K. Shashok (AuthorAID in the Eastern Mediterranean) for improving the manuscript's English use.

List of Abbreviations

NGAL: Neutrophil gelatinase-associated lipocalin
VUR: Vesicoureteral reflux
UTI: Urinary tract infection
ESR: Erythrocyte sedimentation rate

Conflict of Interests

The authors declare that they have no competing interests.

References

1. Doern CD, Richardson SE. Diagnosis of Urinary Tract Infections in Children. Journal of clinical microbiology. 2016;54(9):2233-42.
2. Tullus K, Shaikh N. Urinary tract infections in children. The Lancet. 2020;395(10237):1659-68.
3. Yousefi A, Mohamadian S, Morovati Sharifabadi P, Nakhaei S, Norouzi E. How Does Functional Constipation Affect Growth Status in Children? Iran J Pediatr. 2019;29(2):e85700.
4. Bortnick EM, Nelson CP. Vesicoureteral reflux: current care trends and future possibilities. Urol Clin. 2023;50(3):391-402.
5. Cajigas-Loyola SC, Chow JS, Hayatghaibi S, Iyer RS, Kwon J, Rubesova E, et al. Imaging of Vesicoureteral Reflux: AJR Expert Panel Narrative Review. Am J Roentgenol. 2023.
6. Mattoo TK. Vesicoureteral reflux and reflux nephropathy. Adv Chronic Kidney Dis. 2011;18(5):348-54.
7. Läckgren G, Cooper CS, Neveus T, Kirsch AJ. Management of vesicoureteral reflux: what have we learned over the last 20 years? Front Pediatr. 2021;9:650326.
8. Marakala V. Neutrophil gelatinase-associated lipocalin (NGAL) in kidney injury–A systematic review. Clin Chim Acta. 2022;536:135-41.
9. Romejko K, Markowska M, Niemczyk S. The Review of Current Knowledge on Neutrophil Gelatinase-Associated Lipocalin (NGAL). Int J Mol Sci. 2023;24(13):10470.
10. Zou Z, Chen B, Tang F, Li X, Xiao D. Predictive value of neutrophil gelatinase-associated lipocalin in children with acute kidney injury: A systematic review and meta-analysis. Front Pediatr. 2023;11:1147033.
11. Nakhaei S, Talachian E, Bidari A. Familial Mediterranean fever: unusual age of presentation and the role of genetic diagnosis. Arch Iranain Med. 2005;8(0):56-59.
12. Nasioudis D, Witkin SS. Neutrophil gelatinase-associated lipocalin and innate immune responses to bacterial infections. Med Microbiol Immunol. 2015;204(4):471-9.
13. Seo WH, Nam SW, Lee EH, Je BK, Yim HE, Choi BM. A rapid plasma neutrophil gelatinase-associated lipocalin assay for diagnosis of acute pyelonephritis in infants with acute febrile urinary tract infections: a preliminary study. Eur J Pediatr. 2014;173(2):229-32.
14. Shaikh K, Rajakumar V, Osio VA, Shaikh N. Neutrophil gelatinase-associated lipocalin for urinary tract infection and pyelonephritis: a systematic review. Pediatr Nephrol. 2021;36:1481-7.
15. Horváth J, Wullt B, Naber KG, Köves B. Biomarkers in urinary tract infections–which ones are suitable for diagnostics and follow-up? GMS Infect Dis. 2020;8.
16. Mattoo TK, Spencer JD. Biomarkers for urinary tract infection: present and future perspectives. Pediatr Nephrol. 2024:1-12.
17. Valério FC, Lemos RD, de C Reis AL, Pimenta LP, Vieira ÉL, Silva ACe. Biomarkers in vesicoureteral reflux: an overview. Biomark Med. 2020;14(8):683-96.
18. Prasad MM, Cheng EY. Imaging studies and biomarkers to detect clinically meaningful vesicoureteral reflux. Investig Clin Urol. 2017;58(Suppl 1):S23-s31.
19. Parmaksiz G, Noyan A, Dursun H, Ince E, Anarat R, Cengiz N. Role

[DOI: 10.47176/mjiri.38.59]

of new biomarkers for predicting renal scarring in vesicoureteral reflux: NGAL, KIM-1, and L-FABP. Pediatr Nephrol (Berlin, Germany). 2016;31(1):97-103.
20. Ichino M, Kusaka M, Kuroyanagi Y, Mori T, Morooka M, Sasaki H, et al. Urinary neutrophil-gelatinase associated lipocalin is a potential noninvasive marker for renal scarring in patients with vesicoureteral reflux. The Journal of urology. 2010;183(5):2001-7.
21. Colceriu M-C, Aldea PL, Boț A-L, Bulată B, Delean D, Grama A, et al. The Utility of Noninvasive Urinary Biomarkers for the Evaluation of Vesicoureteral Reflux in Children. Int J Mol Sci. 2023;24(24):17579.
22. Gavrilovici C, Dusa CP, Iliescu Halitchi C, Lupu VV, Spoiala EL, Bogos RA, et al. The role of urinary NGAL in the management of primary vesicoureteral reflux in children. Int J Mol Sci. 2023;24(9):7904.
23. Yilmaz A, Sevketoglu E, Gedikbasi A, Karyagar S, Kiyak A, Mulazimoglu M, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. Pediatr Nephrol (Berlin, Germany). 2009;24(12):2387-92.
24. Decavele AS, Dhondt L, De Buyzere ML, Delanghe JR. Increased urinary neutrophil gelatinase associated lipocalin in urinary tract infections and leukocyturia. Clin Chem Lab Med. 2011;49(6):999-1003.
25. Lee HE, Kim DK, Kang HK, Park K. The diagnosis of febrile urinary tract infection in children may be facilitated by urinary biomarkers. Pediatr Nephrol (Berlin, Germany). 2015;30(1):123-30.
26. Parravicini E, Nemerofsky SL, Michelson KA, Huynh TK, Sise ME, Bateman DA, et al. Urinary neutrophil gelatinase-associated lipocalin is a promising biomarker for late onset culture-positive sepsis in very low birth weight infants. Pediatr Res. 2010;67(6):636-40.
27. Nickavar A, Valavi E, Safaeian B, Moosavian M. Validity of urine neutrophile gelatinase-associated lipocalin in children with primary vesicoureteral reflux. Int Urol Nephrol. 2020;52(4):599-602.
28. Amiri R, Faradmal J, Rezaie B, Sedighi I, Sanaei Z, Solgi G. Evaluation of urinary neutrophil gelatinase-associated lipocalin as a biomarker in pediatric vesicoureteral reflux assessment. Iran J Kidney Dis. 2020;14(5):373.

[DOI: 10.47176/mjiri.38.59]