Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2022;11(1):70-4

Neutrophil gelatinase-associated lipocalin as a biomarker for the diagnosis of urinary tract infection in children

Utku Pamuk[1], Suleyman Kalman[2], Mehmet Emre Tascilar[3], Erdim Sertoglu[4]

[1]Ankara Education and Research Hospital, University of Health Sciences Department of Pediatric Cardiology , Ankara,Turkey
[2]Liv Hospital, Department of Pediatric Nephrology, Ankara, Turkey
[3]Koru Ankara Hospital, Department of Pediatric Endocrinology, Ankara, Turkey
[4]University of Health Sciences, Gülhane School of Medicine, Department of Medical Biochemistry, Ankara, Turkey

Received 09 June 2021; Accepted 22 January 2022
Available online 06.12.2021 with doi: 10.5455/medscience.2021.06.202

Abstract

Urinary tract infection (UTI) is common in childhood and may have important consequences. The reference standard for the diagnosis is urine culture, but it requires time for bacterial growth. Neutrophil gelatinase-associated lipocalin (NGAL) has been shown to be elevated after kidney injury. This study aims to investigate the usefullness of blood and urine NGAL levels in children with UTI. Fifty-nine patients with UTI (29 with pyelonephritis, 30 with lower UTI) and 28 healthy controls were enrolled in the study. NGAL values were determined by using ELISA. Urine NGAL and urine NGAL/creatinine levels were significantly higher in patients with UTI than healthy controls ($p<0.001$) as well as in patients with pyelonephritis than in patients with lower UTI ($p=0.016$ for urine NGAL, $p=0.001$ for urine NGAL/creatinine). Besides, plasma NGAL levels were found to be higher in patients with pyelonephritis than patients with lower UTI ($p=0.015$) and healthy controls ($p=0.016$). In conclusion, urine NGAL and urine NGAL/creatinine can be used for the detection of UTI and the differential diagnosis of pyelonephritis and lower UTI. Plasma NGAL can also be useful for determining pyelonephritis in children.

Keywords: VNGAL, children, Neutrophil gelatinase associated lipocalin, Lipocalin, urinary tract infection

Introduction

Urinary tract infection (UTI) is a common bacterial infection in childhood, which may have important consequences such as scarring of the kidney and renal failure [1]. The decision to start treatment is made by urinalysis and urine microscopy, but the reliability of these tests is not sufficient for diagnosis [2, 3]. The negative predictive value of the leukocyte esterase test is not satisfactory, and the nitrite test and urine microscopy did not increase this [3]. The reference standard for the diagnosis is urine culture, but it requires at least 18 hours for bacterial growth [4]. At the same time, differential diagnosis of upper and lower UTI is required for the treatment and follow-up process of patients, but this distinction is not always clear to see [1, 2].

Neutrophil gelatinase-associated lipocalin (NGAL) is a newly defined protein found in human neutrophil granules and associated with the immune system [5, 6]. It has been found to be secreted from the renal tubules after kidney damage [7]. Plasma NGAL levels were found to be high in children and adolescents with chronic renal failure undergoing dialysis [8]. It has also been shown to be increased in mice with chronic renal failure [9]. Mussap et al. showed that the patients with acute kidney damage in the intensive care unit can be detected much earlier with NGAL in comparison to serum creatinine [10]. In the urine of mice with pyelonephritis, NGAL levels were found to rise early [11]. Also, urinary NGAL levels were found to be high in UTI in pediatric and adult patients [12, 13].

This study aims to evaluate whether blood and urine NGAL levels can be used in the diagnosis of UTI and to determine their usability in the distinction of lower urinary tract infection and pyelonephritis.

Materials and Methods

Fifty-nine patients, diagnosed UTI, and 28 healthy children of similar age and gender were enrolled in the study admitted to outpatient clinic between January 2011 and March 2012 prospectively.

Urine samples was obtained by urethral catheterization in children

***Corresponding Author:** Utku Pamuk, Ankara Education and Research Hospital, University of Health Sciences Department of Pediatric Cardiology, Ankara,Turkey
E-mail: drgulpmk@gmail.com

unable to control voiding and midstream technique in older children. Urinalysis, urine microscopy, urine culture, urine creatinine, hemogram, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) tests were performed in the patients and the control group. Urinary tract infection was defined if there was significant bacterial growth in urine culture (≥105 cfu/ml from midstream urine or ≥104 cfu/ml from catheterized urine). Pyelonephritis was defined as persistently high fever > 38.5 ˚C, increased CRP, ESR >20 mm/h and a positive urine culture primarily based on the UTI guideline from the American Academy of Pediatrics [14].

To analyse urine and blood NGAL, urine and blood samples taken at the time of presentation were centrifuged for 10 minutes at 4000 rpm. Supernatants of urine samples and serums of blood samples were stored at -80 ºC. Urine and plasma NGAL was determined using human Lipocalin-2/NGAL enzyme-linked immunosorbent assay (ELISA) kit (Cat no: RD191102200R) purchased from BioVendor Research and Diagnostic Products (Czech Republic).

Plasma NGAL, urine NGAL and urine NGAL/creatinine values of patients with pyelonephritis, lower UTI and control group were compared.

All statistical analyzes of the study were performed with SPSS 15.0. Since numerical variables do not show normal distrubution, they are specified as median and interquartile range. Comparisons between multiple groups were made using Kruskal-Wallis test, and comparisons between the groups were made using the Mann-Whitney U test. Optimal cutoff values of plasma and urine NGAL and urine NGAL/creatinine for UTI were investigated using ROC analysis. The area under the ROC curve (AUC) with a 95% confidence interval (CI), sensitivity, and specificity were calculated. Statistical significance was set at $p<0.05$.

The study was approved by local ethics committee, and informed consents were obtained from all attendants.

Results

The patient group involved 59 children (47 female, 12 male) with UTI, and the mean age was 6.42±4.47 years (1 month to 16 years). The control group consisted of 28 healthy children (21 female, seven male), and the mean age was 5.59±4.68 years (2 months to 15 years). Twenty-nine of the 59 patients (49.1%) were diagnosed with pyelonephritis and the other 30 (50.9%) had lower UTI.

ESR and CRP levels were higher in pyelonephritis group than lower UTI and control group (Table 1). ESR levels also were higher in lower UTI group than control group. The most common cause for UTI in two groups was Esherichia coli.

Median plasma NGAL level of the patients with pyelonephritis was significantly higher than the patients with lower UTI (89.5 ng/ml vs 62.1 ng/ml, p=0.015) and healthy controls (89.5 ng/ml vs 65.7 ng/ml, p=0.016) (Table 2, Fig 1).

Patients with pyelonephritis had higher median urine NGAL level than the patients with lower UTI (66.6 ng/ml vs 30.4 ng/ml, p=0.016) and controls did (66.6 ng/ml vs 4.5 ng/ml, p<0.001) (Table 2, Fig 1). In addition, median urine NGAL level of the patients with lower UTI was significantly higher than healthy controls (p<0.001). Median urine NGAL/creatinine level of the patients with pyelonephritis was found to be higher than the patients with lower UTI (1.67 ng/ml vs 0.48 ng/ml, p=0.001) and controls (1.67 ng/ml vs 0.07 ng/ml, p<0.001) (Table 2, Fig 1). In addition, the patients with lower UTI had higher median urine NGAL/creatinine level than controls (p<0.001).

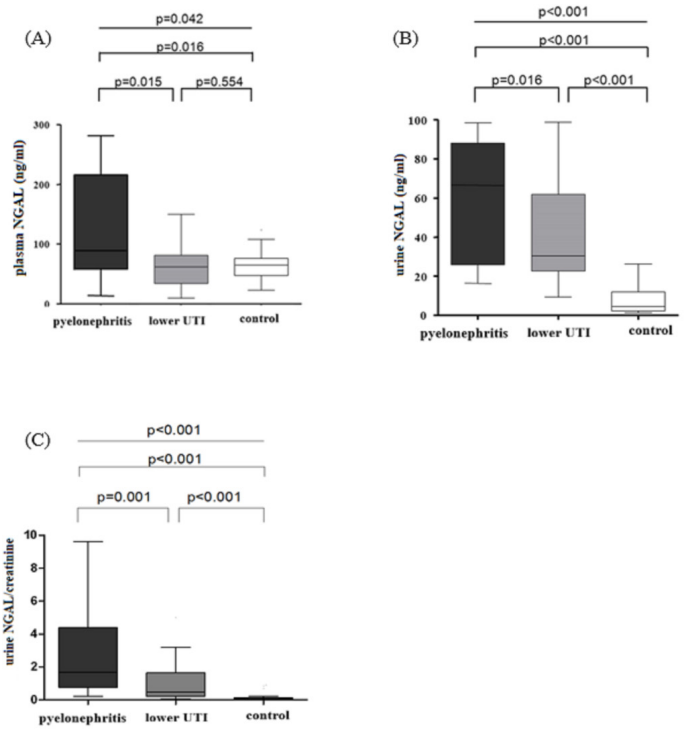

Figure 1. Levels of plasma (A), urine NGAL (B) and urine NGAL/creatinine (C) in patients with pyelonephritis, lower UTI and the healthy control group. Horizontal lines represent the medians, boxes represent the interquartile range (25-75%).

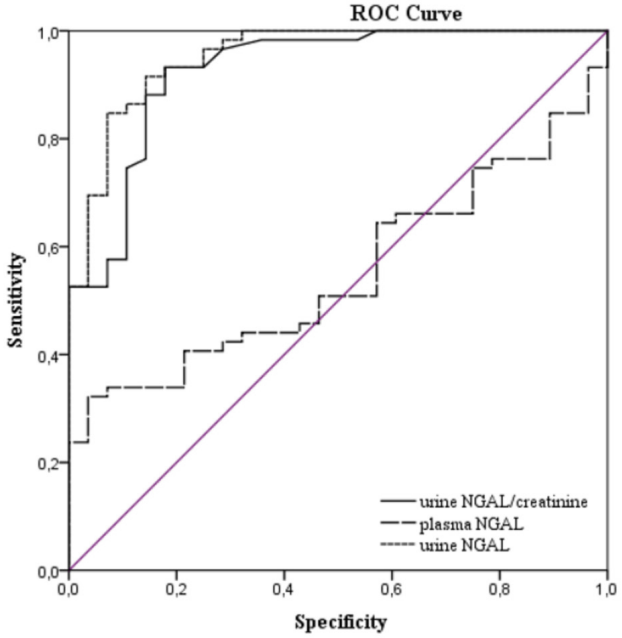

Figure 2. Receiver operation characteristic (ROC) curves to detect UTI.

There is a positive correlation between ESR with serum and urine NGAL and urine NGAL/ceratinine levels ($p < 0.05$). CRP levels

were also corralete with urine NGAL and NGAL/creatinine levels ($p < 0.05$).

ROC analyses were used to describe diagnostic profiles of plasma and urine NGAL and urine NGAL/creatinine. The optimal cutoff level of plasma NGAL was 37.1 ng/ml with 86% sensitivity and 79% specificity, and the AUC value was 0.659 (CI 0.524-0.795) to predict pyelonephritis (Fig. 2). Urine NGAL AUC value was 0.952 (CI 0.908- 0.997) and the optimal cutoff value was 15.6 ng/ml with 92% sensitivity and 86% specificity, and the optimal urine NGAL/creatinine cutoff level was 0.37 with sensitivity 90 %, specificity 65%, and the AUC value was 0.853 (CI 0.775-930) to predict UTI. The optimal cutoff value of urine NGAL was 16.8 ng/ml with 90% sensitivity and 90% specificity and the AUC value was 0.815 (CI 0.725-0.904), and urine NGAL/creatinine AUC value was 0.926 (CI 0.867-0.986) and the optimal cutoff value was 0.17 to predict pyelonephritis. Using a cutoff of 0.17 for urine NGAL/creatinine for the diagnosis of pyelonephritis, sensitivity and specificity were 93% and 82% respectively.

Table 1. Clinical findings of patients and control group

	Pyelonephritis (n=29)	lower UTI (n=30)	control (n=28)	p
CRP	57.3±3.1	3.1±0.6	1.6±0.2	0.000
ESR	67.3±3.9	13.3±0.9	6.7±0.7	0.000
Germ of UTI				
Escherichia coli	25	23		
Klebsiella pneumonia	3	3		0.506
Proteus mirabilis	1	3		
Enterococcus faecalis		1		

CRP= C reactive protein, ESR = erythrocyte sedimentation rate, UTI = urinary tract infection

Table 2. Plasma, urine NGAL and urine NGAL/creatinine levels of the patient and control groups

	Plasma NGAL (ng/ml)	p	Urine NGAL (ng/ml)	p	Urine NGAL/creatinine (ng/ml)	p
Pyelonephritis (n=29)	89.5 (58.5-216.1)		66.6 (25.7-88)		1.67 (0.75-4.38)	
Lower UTI (n=30)	62.1 (34.7-81)	0.042	30.4 (22.6-62)	<0.001	0.48 (0.22-1.64)	<0.001
Control (n=28)	65.7 (48-77)		4.5 (2.3-12)		0.07 (0.02-0.14)	

Note: Data were presented as median (25%-75%)

Discussion

Urinary tract infection is common among children and associated with long-term morbidity. Early diagnosis and treatment of urinary tract infection is important in terms of preventing complications such as renal scar, hypertension, and end-stage renal failure. Urine culture is gold standard in the diagnosis of UTI, but at least 18 hours are required to detect any bacterial growth [4]. In daily practice, antibiotic treatment is started in line with urinalysis and urine microscopic examination, but it is stated that the sensitivity and specificity of these tests are not sufficient [15-17].

NGAL is an iron binding protein originating from neutrophil and epithelial cells. Previous studies have reported that NGAL is released from neutrophils during infection, binds the iron that bacteria need and shows a bacteriostatic effect [9, 11]. NGAL has been found to be secreted from the renal tubules after kidney damage, and increased in the urine in a short time after injury [7]. Urine NGAL level has been associated with the severity and residual kidney function in various chronic kidney diseases such as autosomal polycystic kidney disease, systemic lupus erythematosus, IgA nephropathy, diabetic nephropathy, and idiopathic glomerulonephritis [18, 19]. In addition, urinary NGAL level has been determined to be significant to ascertain kidney damage in patients admitted to the pediatric emergency department and children after congenital heart surgery [20, 21].

In this study, patients with pyelonephritis had higher plasma NGAL, urinary NGAL and urinary NGAL/creatinine levels than the patients with lower UTI and healthy controls. It is also found the levels of urinary NGAL and NGAL/creatinine were higher in the patients with lower UTI than the healthy controls.

In early studies, it was stated that mice with UTI had high urinary NGAL levels [11, 13]. Recent research has shown special emphasis has been given to the relationship between UTI and NGAL in humans. Hatipoğlu et al. found higher urinary NGAL levels in patients with UTI in comparison to healthy children [12]. Another study displayed higher levels of urine NGAL and NGAL/creatinine in children with UTI, as well as the patients with pyelonephritis, compared to the patients with lower UTI [13]. Also, they reported that urine NGAL levels above 20 ng/ml were significant in the diagnosis of UTI. In our study, a close cutoff value as 15.6 ng/ml for urine NGAL has been found in the diagnosis of UTI. Likewise, our results have demonstrated elevated levels of urine NGAL and urine NGAL/creatinine in patients with UTI. Furthermore, in similar way, we found higher levels of urine NGAL in patients with pyelonephritis than in those with lower UTI but the cut-off values were quite similar. We thought this was due to the low level of specificity of the cut-off value of the pyelonephritis group. In

a recent study, it was stated that urinary NGAL level was higher in febrile infants with UTI, and when low levels were found, it was more likely to have another cause of fever outside the urinary tract [22]. Few studies have suggested that NGAL is not useful in diagnose of UTI [23].

NGAL released from tubules after renal damage has been shown to be reabsorbed through the kidneys and can then be detected in the blood [24]. Plasma NGAL levels have been found to be high in adult and pediatric patients who developed acute renal failure in intensive care units [24, 25]. It has also been found that plasma NGAL levels increased in the early stages in patients with kidney transplantation with graft damage [25]. In patients with acute kidney injury after pediatric cardiac surgery, plasma NGAL levels have been reported to increase as well as urinary NGAL levels [21]. There have been few studies investigating plasma NGAL in patients with UTI in the current literature. Kim et al. suggested that the elevation in plasma NGAL level was meaningful in patients with acute pyelonephritis and values above 117 ng/ml were significant for the diagnosis [26]. In the current study, plasma NGAL levels were found to be higher in patients with acute pyelonephritis as in the previous study; however a lower cutoff level of 37 ng/ml with a low specifity was determined. Supporting these findings, another recenly published study found that plasma NGAL levels were higher in patients with pyelonephritis, especially in those with renal cortical defects [27]. However, we did not find a significant difference when we compared the patients with lower UTI and healthy controls in terms of plasma NGAL level. This may be due to the absence of kidney damage in lower UTI or the absence of inflamation. There are several publications in the literature suggesting that plasma NGAL level may increase secondary to inflamation and urinary NGAL is more specific in terms of pyelonephritis [28].

Conclusion

Both urinary and plasma NGAL has been the subject of many types of research in the diagnosis of UTI in which it was mostly found to be a powerful predictor. The current study has shown urine NGAL and urine NGAL/creatinine levels may provide early diagnosis of UTI. It is also determined that they can be used in the distinction of pyelonephritis and lower UTI. The study also revealed that higher plasma NGAL levels in patients may predict pyelonephritis. Our urine and plasma cut-off values for pyelonephritis have low specificity. Larger studies may confirm the use of urine and plasma NGAL as a biomarker in the setting of infection and find optimal cut-off values.

Conflict of interests
The authors declare that they have no conflicts of interest concerning this article.

Financial Disclosure
All authors have read and approved the final form of this article.

Ethical approval
The local ethics committee approved the study, (1491-1188-10/1539, 8/12/2010)

References

1. Hansson S, Urinary tract infection, Pediatric Nephrology. 1999;835-50.
2. Jantausch B. Urinary tract infections, Clinical Pediatric Nephrology 2007:553-74.
3. Nostrand JD Van, Junkins AD, Bartholdi RK, Poor predictive ability of urinalysis and microscopic examination to detect urinary tract infection, Am J Clin Pathol. 2000;113:709-13.
4. Williams GJ, Craig JC. Diagnosis and management of urinary tract infections, Comprehensive Pediatric Nephrology, Elsevier. 2008;539-48.
5. Nguyen MT, Devarajan P. Biomarkers for the early detection of acute kidney injury. Pediatric Nephrol.2008;23;2151-7.
6. Xu S, Carlson M, Engström A, et al. Purification and characterization of a human neutrophil lipocalin (HNL) from the secondary granules of human neutrophils, Scandinavian journal of clinical and laboratory investigation 1994;54:365-76.
7. Langelueddecke C, Roussa E, Fenton RA, et al. Thévenod, Lipocalin-2 (24p3/neutrophil gelatinase-associated lipocalin (NGAL)) receptor is expressed in distal nephron and mediates protein endocytosis, J Biol Chem. 2012;287:159-69.
8. Musiał K, Zwolińska D, Neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteinases as novel stress markers in children and young adults on chronic dialysis. Cell Stress Chaperones. 2011;16:163-71.
9. Viau A, El Karoui K, Laouari D, et al. Lipocalin 2 is essential for chronic kidney disease progression in mice and humans. J Clin Investigation. 2010;120:4065-76.
10. Mussap M, Degrandi R, Fravega M, Fanos V. Acute kidney injury in critically ill infants: the role of urine Neutrophil Gelatinase-Associated Lipocalin (NGAL). J Maternal-Fetal Neonatal Med. 2010;23(sup3):70-2.
11. Ichino M, Kuroyanagi Y, Kusaka M, et al. Increased urinary neutrophil gelatinase associated lipocalin levels in a rat model of upper urinary tract infection. J Urology. 2009;181:2326-31.
12. Hatipoglu S, Sevketoglu E, Gedikbasi A, et al. Urinary MMP-9/NGAL complex in children with acute cystitis. Pediatric Nephrol. 2011;26:1263-8.
13. Yilmaz A, Sevketoglu E, Gedikbasi A, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. Pediatric Nephrol. 2009;24:2387.
14. Subcommittee on Urinary Tract Infection SCoQI, Management. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Am Acad Pediatrics; 2011.
15. Arinzon Z, Peisakh A, Shuval I, et al. Detection of urinary tract infection (UTI) in long-term care setting: Is the multireagent strip an adequate diagnostic tool? Arch Gerontol Geriatrics. 2009;48:227-31.
16. Santos JCd, Weber LP, Perez LRR. Evaluation of urinalysis parameters to predict urinary-tract infection. Brazilian J Infectious Diseases. 2007;11:479-81.
17. Taneja N, Chatterjee S, Singh M, Sivapriya S, Sharma M, Sharma S. Validity of quantitative unspun urine microscopy, dipstick test leucocyte esterase and nitrite tests in rapidly diagnosing urinary tract infections. J Assoc Physicians India. 2010;58:485-7.
18. Nielsen S, Schjoedt K, Astrup A, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 (KIM1) in patients with diabetic nephropathy: a cross-sectional study and the effects of lisinopril. Diabetic Med. 2010;27:1144-50.
19. Soni SS, Cruz D, Bobek I, Chionh CY, Nalesso F, Lentini P, et al. NGAL: a biomarker of acute kidney injury and other systemic conditions. Intern Urology Nephrol. 2010;42:141-50.
20. Du Y, Zappitelli M, Mian A, et al. Urinary biomarkers to detect acute kidney injury in the pediatric emergency center. Pediatric Nephrol. 2011;26:267-74.
21. Parikh CR, Devarajan P, Zappitelli M, et al. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. J Am Society Nephrol. 2011;22:1737-47.
22. Valdimarsson S, Jodal U, Barregård L, Hansson S. Urine neutrophil gelatinase-associated lipocalin and other biomarkers in infants with urinary tract infection and in febrile controls. Pediatric Nephrology. 2017;32:2079-87.
23. Kim BH, Yu N, Kim HR, et al. Evaluation of the optimal neutrophil gelatinase-associated lipocalin value as a screening biomarker for urinary tract infections in children. Ann Laboratory Med. 2014;34:354-9.

24. Wheeler DS, Devarajan P, Ma Q, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Critical Care Med. 2008;36:1297.

25. de Geus HR, Woo JG, Wang Y, et al. Urinary neutrophil gelatinase-associated lipocalin measured on admission to the intensive care unit accurately discriminates between sustained and transient acute kidney injury in adult critically ill patients. Nephron Extra. 2011;1:9-23.

26. Kim BK, Yim HE, Yoo KH. Plasma neutrophil gelatinase-associated lipocalin: a marker of acute pyelonephritis in children. Pediatric Nephrology. 2017;32:477-84.

27. Seo WH, Nam SW, Lee EH, Je B-K, 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 Pediatric. 2014;173:229-32.

28. Forster CS, Devarajan P. Neutrophil gelatinase-associated lipocalin: utility in urologic conditions. Pediatric Nephrology. 2017;32:377-81.