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ORIGINAL ARTICLE

Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin

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Abstract Neutrophil gelatinase associated lipocalin (NGAL) is a protein identified in human neutrophil granules. The aim of the study was to assess whether urine level of NGAL (uNGAL) could represent a novel, reliable marker of urinary tract infection (UTI) and to determine the optimal cutoff level for uNGAL to predict UTI in children. Sixty patients with symptomatic UTI and 29 healthy controls were enrolled the study. Urine NGAL was measured by enzyme-linked immunosorbent assay. A dimercaptosuccinic acid (DMSA) radionuclide scan was performed within 7 days in the patients with UTI in an attempt to distinguish pyelonephritis from cystitis. Mean uNGAL level was significantly higher in the UTI group than in the controls (91.02 ng/ml vs 14.29 ng/ml, $p=0.0001$) and using a cutoff 20 ng/ml for uNGAL for diagnosis of UTI, sensitivity, and specificity were 97% and 76%, respectively [area under the curve (AUC): 0.979]. Mean uNGAL/creatinine ratio (uNGAL/Cr) was also significantly higher in the UTI group [201.81 ng/mg creatinine (Cr) vs 18.08 ng/mg Cr; $p=0.0001$], and using a cutoff 30 ng/mg Cr for uNGAL/Cr for diagnosis of UTI, sensitivity and specificity were 98% and 76%, respectively (AUC: 0.992). In conclusion, both uNGAL and uNGAL/Cr can be used as a novel, sensitive marker for early prediction of UTI in the absence of acute kidney injury and chronic kidney disease, and the optimal cutoff value for prediction of UTI is lower than the values determined for acute kidney injury. Further investigations with larger patient groups are required to confirm our results.

Keywords Urinary tract infection · Neutrophil · Lipocalin · Neutrophil gelatinase associated lipocalin · NGAL · Human neutrophil lipocalin · HNL · Child

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Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections in children [1, 2]. Early diagnosis and treatment of UTI is important, because missed or delayed diagnosis of UTI may result in the failure of appropriate treatment and possibly lead to long-term consequences, including renal scarring, hypertension, and chronic renal failure [1]. Although urine culture is the gold standard for diagnosing UTI [2], positive culture results require 2–3 days to complete identification of the bacteria found. Therefore, in daily practice, urinalysis is commonly used to diagnose UTI and to decide whether to start treatment. As indicators

of UTI, pyuria and positive nitrite test in urinalysis have some limitations [2]. One of the limitations is that sterile pyuria may occur in other noninfectious conditions such as urolithiasis [2]. On the other hand, it is well known that pyuria may be absent in infections with *Proteus* species [2]. Similarly, nitrite tests cannot detect the presence of gram-positive pathogens and also high urine-specific gravity decreases the sensitivity of this test [2]. We consider that another marker for rapid and accurate diagnosis of UTI would be valuable for early initiation of treatment in children with suspected UTI pending urine culture result.

Neutrophil gelatinase associated lipocalin (NGAL) is a novel protein identified in human neutrophil granules and is considered to be a component of the innate immune system [3–6]. In animal studies, it has been demonstrated that NGAL-deficient mice have increased susceptibility to infection with *Escherichia coli* and death with sepsis [4–6]. Serum level of NGAL has been measured in human studies, and it has been reported that serum NGAL level is a useful marker to distinguish acute bacterial from viral infection in newborns, children, and adults [7–9]. Recently, Ichino et al. [10] constituted an experimental pyelonephritis in rats and established that urine level of NGAL in rats increased in the early stage of UTI, suggesting that urine NGAL (uNGAL) might be used to predict UTI in humans as well.

The aim of our study was to assess whether uNGAL could represent a novel, sensitive marker of UTI and to determine the optimal cutoff level for uNGAL to predict UTI in children.

Patients and methods

This multicenter, prospective study was conducted over a 1-year period at three research and training hospitals between March 2008 and March 2009 in Turkey. Sixty consecutive patients who presented to our outpatient clinic with symptomatic UTI (UTI group) and 30 healthy children (control group) were enrolled in the study, but one of healthy children was excluded from the study because of bacterial growth in his urine. Symptoms of the patients at the time of presentation are shown in Table 1. After obtaining informed consent, urine culture, urinalysis, serum urea, and serum creatinine (Cr), C-reactive protein (CRP), and serum white blood cell count measurements were performed in both groups at the time of presentation. Leukocytosis was defined as leukocyte count more than normal value according to age [11], and positive CRP was defined as CRP value >5 mg/L.

Urine samples for culture were obtained by collecting bag or midstream urine in the control group. The children in the control group were excluded from the study if they had bacterial growth in their urine. Urine samples were obtained by catheter in patients who presented with symptoms suggesting UTI, such as fever, loin pain, and voiding frequency, and UTI was diagnosed if there was significant bacteriuria (≥10,000 cfu/ml) in the urine culture [1]. Urinalysis including leukocyte esterase reaction, nitrite test, and microscopic urine analysis was performed by Iris IQ 200 fully automatic urine analyzer.

Table 1 Complaints of the patients at the time of presentation

	Number (%)
Presence of upper UTI symptoms	
Fever	19 (31.6)
Loin pain	6 (10)
Abdominal pain	13 (21.6)
Nausea/vomiting	7 (11.6)
Hematuria	2 (3.3)
Agitated baby	8 (13.3)
Presence of lower UTI symptoms	
Dysuria	11 (18.3)
Voiding frequency	5 (8.3)
Urinary incontinence	10 (16.6)
Pungent odor in urine	1 (1.6)

UTI urinary tract infection

Random urine samples were obtained for measuring uNGAL and Cr from both groups at the time of presentation and prior to treatment. The urine samples were immediately centrifuged at 4°C for 15 min at 13,000 *g*. Aliquots of urine supernatant were stored at –80°C for assaying. Urine NGAL was determined using human NGAL/lipocalin-2 enzyme-linked immunosorbent assay (ELISA) kit (Cat no: CY-8070), purchased from CircuLex (Tera- Sawaoka, Japan) following the manufacturer's instructions. CircuLex's NGAL/lipocalin-2 ELISA employs the quantitative sandwich enzyme immunoassay technique. NGAL levels were expressed as nanograms per milliliter.

All children in the UTI group were investigated with a dimercaptosuccinic acid (DMSA) scan within 7 days in an attempt to distinguish acute pyelonephritis from cystitis. DMSA scan was performed 2 h following the intravenous injection of 2 MBq/kg (minimum 15 MBq, maximum 100 MBq) Tc^{99m}-DMSA (MON.DMSA kit, Monrol, Turkey). All patients were imaged on a dual-head gamma camera equipped with a low-energy (140 keV±20%), high-resolution, parallel-hole collimator (E-cam, Siemens, Chicago, IL, USA). Posterior, left posterior, and right posterior oblique planar images were obtained in a 128 × 128 matrix for a minimum of 500,000 counts each. The patients with normal scintigraphy were considered as having lower UTI. Scan features to suggest acute pyelonephritis included

focal, multifocal, or diffusely decreased or absent cortical uptake without cortical volume loss, in which the renal cortical contour remains intact. When scintigraphy revealed renal parenchymal lesions, a second scintigraphy was performed at least 6 months later to evaluate the progression of renal lesions. On the follow-up scan, we considered every persisting lesion as a scar in the absence of UTI [12]. Diagnosis of acute pyelonephritis was confirmed only in patients with totally or partially reversible lesions on DMSA scan. This study was approved by the local ethics committee and performed according to the ethical standards of the Declaration of Helsinki.

Statistical calculations were performed with NCSS 2007 program for Windows. Besides standard descriptive statistical calculations (mean, standard deviation, median, and geometric mean), one-way analysis of variance (ANOVA) was used to compare groups, post hoc Tukey multiple comparison test was utilized to compare subgroups, unpaired *t* test was used to compare two groups, and the chi-square test was performed during the evaluation qualitative data. The results were evaluated within a 95% confidence interval (CI). Urine NGAL and uNGAL/Cr were tested for their normal distribution. Logarithmic transformations were applied as needed to achieve a distribution to normal. We used geometric mean uNGAL and uNGAL/Cr. Receiver operating curve (ROC) analysis was performed to determine sensitivity and specificity of different cutoff points for uNGAL and uNGAL/Cr to predict UTI. The most appropriate cutoff point was chosen according to ROC analysis, and the area under the curve (AUC) was calculated. Statistical significance level was established at $p<0.05$.

Results

The UTI group consisted of 60 children (52 female, eight male), and the mean age was 5.95±3.84 years (2 months to 12 years). The control group consisted of 29 healthy children (13 female, 16 male), and mean age was 6.67± 4.52 years (3 months to 14 years). Serum urea and Cr levels were normal in all children in the UTI and control groups. Mean uNGAL level was significantly higher in the UTI group than in controls (91.02 ng/ml vs 14.29 ng/ml, $p=0.0001$) (Table 2, Fig. 1). According to ROC analysis, the optimal cutoff level was 20 ng/ml for uNGAL to predict UTI. Using a cutoff of 20 ng/ml for uNGAL for diagnosis of UTI, sensitivity and specificity were 97% and 76%, respectively (Fig. 2). The positive and negative predictive values of this cutoff point were 89% and 92%, respectively. When uNGAL is higher than this cutoff value, the possibility of UTI increases four times (positive likelihood ratio 4 and negative likelihood ratio 0.04).

Mean uNGAL/Cr was also significantly higher in the UTI group than in the controls (201.81 ng/mg Cr vs 18.08 ng/mg Cr; $p=0.0001$) (Table 2, Fig. 1). Using a cutoff of 30 ng/mg Cr for uNGAL/Cr for diagnosis of UTI, sensitivity and specificity were 98% and 76%, respectively (Fig. 2). The positive and negative predictive values were 89% and 92%, respectively. When uNGAL/Cr is higher than this cutoff value, the possibility of UTI increases four times (positive likelihood ratio 4.07 and negative likelihood ratio 0.02). AUC was 0.979 for uNGAL and 0.992 for uNGAL/Cr, suggesting that both uNGAL and uNGAL/Cr were excellent markers to diagnose UTI in children.

Sixteen of 60 patients in the UTI group had parenchymal lesions on the first DMSA scan. Second scintigraphy revealed that four patients had interval resolution of the initial abnormal findings, suggesting acute pyelonephritis. On the other hand, 12 patients (20%) had persistent abnormalities, suggesting renal injury. Seven (58.3%) patients with scars had a prior history of UTI, and two of them (28.5%) had additional UTI in the time between the two DMSA scans. Mean uNGAL was higher in patients with renal scar than in patients with normal DMSA scan ($p<0.05$) (Table 2), whereas mean uNGAL/Cr was not different in those two groups ($p>0.05$) (Table 2). ROC analysis could not be performed for patients with acute pyelonephritis and renal scar because of the limited number of subjects.

Urine NGAL and uNGAL/Cr were not correlated to positive serum CRP, positive urine nitrite test, pyuria, urine pH, and specific gravity ($p>0.05$). Urine NGAL was not related to leukocytosis in serum and leukocyte esterase reaction in urine, whereas uNGAL/Cr was significantly higher in patients with 2(+) and 3(+) leukocyte esterase reaction in the urine as well as in patients with leukocytosis in serum ($p<0.05$).

Microorganisms established in urine culture were *E. coli* in 47 patients, *Klebsiella* in seven, *Proteus* in three, *Enterobacter* in two, and *Pseudomonas* in one. Extended spectrum beta lactamase activity (ESBL) was positive in 11 patient and negative in 49. Urine NGAL and uNGAL/Cr were not significantly different between the patients suffering UTI with ESBL-positive and -negative microorganisms ($p>0.05$).

Voiding cystourethrogram was performed in 31 patients with UTI, and 11 of them had vesicoureteral reflux (VUR). Urine NGAL and uNGAL/Cr were not significantly different in the patients with or without VUR ($p>0.05$). The severity of VUR was not correlated with DMSA findings ($p>0.05$).

Discussion

Neutrophil gelatinase associated lipocalin, an iron-carrier protein derived from human neutrophils, has an important

Table 2 Urine neutrophil gelatinase associated lipocalin (NGAL) and urine NGAL/creatinine levels in urinary tract infection (UTI) and control groups

	Urine NGAL (ng/ml) geometric mean (range)	*P* value	Urine NGAL/creatinine (ng/mg) geometric mean (range)	*P* value
Control group (*n*=29)	14.29 (6.31–39.08)	0.0001	18.08 (5.40–73.5)	0.0001
UTI group (*n*=60)	91.02 (19.72–174.14)		201.81 (24.86–1667.9)	
Lower UTI (*n*=44)	83.75 (19.72–167.78)	<0.05	202.68 (24.86–1227.95)	>0.05
Renal scarring (*n*=12)	127.72 (42.58–174.14)		183.6 (96.32–533.90)	
Acute pyelonephritis (*n*=4)	82.39 (29.32–121.12)	*	255.74 (146.63–712.5)	*

*Insufficient number of patients for statistical analysis

role in the innate immune response to bacterial infection [3, 5]. During infection, bacteria require iron for metabolism and growth within the host [5]. It has been demonstrated that NGAL is released by activated neutrophils of the infected host and prevents bacterial iron uptake consuming the ambient iron and thus reduce bacterial growth [5, 6]. Recent studies revealed that uNGAL is increased in acute kidney injury (AKI) induced by nephrotoxins or ischemia, and it has been speculated that NGAL expression was induced in order to contribute to tissue regeneration after kidney damage [10, 13]. Similarly, expression of NGAL increases as a part of the immune response to remove bacteria in the early stage of infection.

A previous report disclosed that serum level of NGAL increased in patients with acute bacterial infections, including UTI [7], whereas increased levels of uNGAL have only been demonstrated in a UTI rat model [10]. In this rat model of pyelonephritis, *E. coli* was injected in the renal cortex and uNGAL levels peaked within 1 week following injection [10]. This data showed that peak uNGAL level was seen in the early stage of infection. Therefore, we aimed to assess whether an increased level of uNGAL might represent a sensitive marker of UTI in children. The urine test was preferred because obtaining urine is easy and less painful for the child. As a result, we confirmed that uNGAL increased in children with UTI and determined the optimal cutoff level for uNGAL to predict UTI in children. We also demonstrated that increased uNGAL and uNGAL/Cr were not related to positive urine nitrite test, pyuria, positive ESBL activity of the bacteria, or VUR.

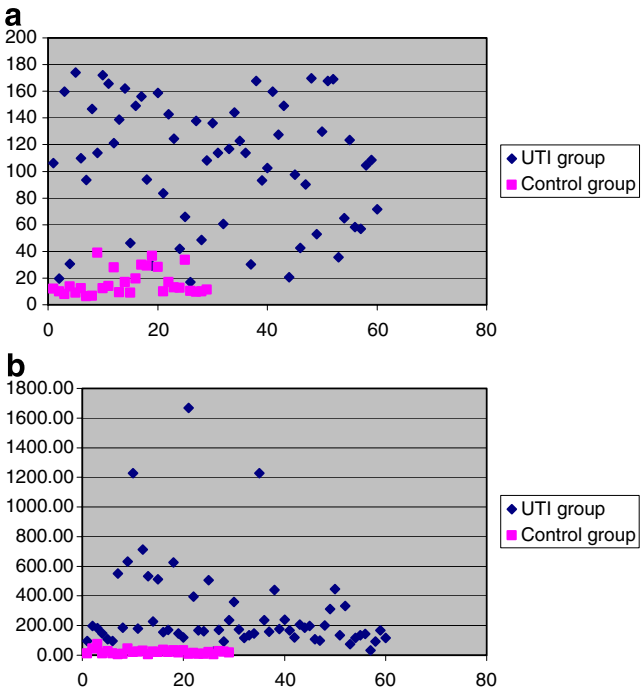

Fig. 1 The distribution of urine neutrophil gelatinase associated lipocalin (uNGAL) levels in urinary tract infections (UTI) and control groups. **a** For uNGAL (ng/ml). **b** For uNGAL/creatinine (ng/mg)

UTI is one of the most common infectious diseases encountered by pediatric healthcare providers. Although the most accurate method for diagnosing UTI is urine culture, results may not be available for 2–3 days. A major concern of the pediatrician is to decide whether antibiotic treatment should be started or not at the time of presentation, as delayed treatment may increase the risk of renal injury. Hence, urinalysis is commonly used to diagnose UTI and the help determine whether early treatment should be initiated. As indicators of UTI, sensitivities of pyuria and positive nitrite tests were found to be low. Arinzon et al. [14] reported that positive leukocyte esterase reaction and nitrite tests are not reliable indicators of UTI due to high rates of false negative results. Another study showed that sensitivity and specificity of leukocyte esterase were 65.4% and 94%, respectively, whereas sensitivity and specificity of the nitrite test were 38.9% and 99.5%, respectively [15]. In this study, we demonstrated that uNGAL can be used to predict UTI. We also determined the optimal cutoff points for uNGAL (20 ng/ml) and uNGAL/Cr (30 ng/mg) in patients with normal renal function. In those determined cutoff points, sensitivity of uNGAL (97%) and uNGAL/Cr (98%) were found to be higher than in leukocyte esterase or the nitrite test, although specificity of uNGAL (76%) and uNGAL/Cr (76%) was lower than in urinalysis. Using

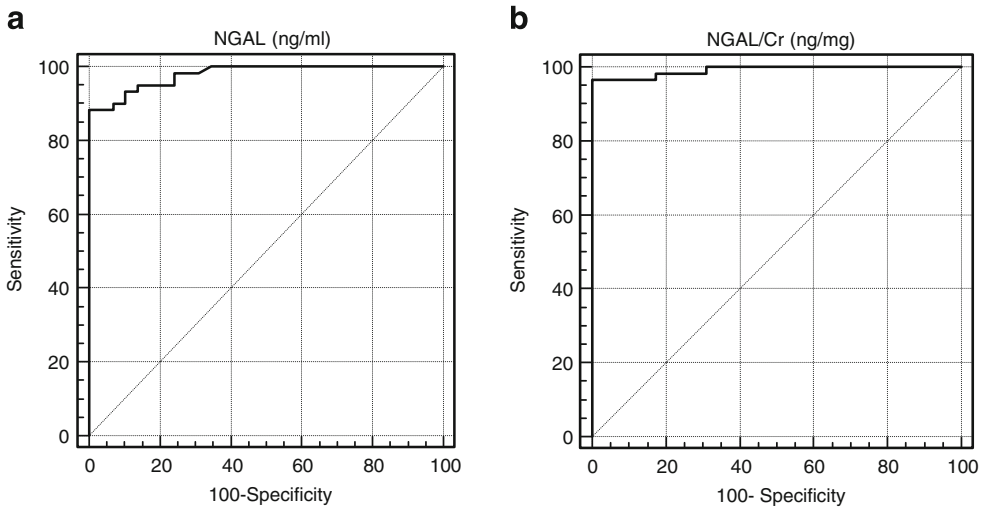

Fig. 2 Receiver operating curve to detect urinary tract infection (UTI). **a** For urine neutrophil gelatinase associated lipocalin (uNGAL) [area under the curve (AUC)=0.979]. **b** For uNGAL/creatinine (AUC=0.992)

optimal cutoff values, treatment can be started before urine culture results are obtained.

Urine NGAL has been reported to be increased in acute kidney injury (AKI) [6, 13, 16–18]. Hirsch et al. [13] reported that AKI due to contrast administration can be predicted using a cutoff of 100 ng/ml for uNGAL. In different studies, the cutoff values for predicting AKI after cardiopulmonary bypass were determined as 50 ng/ml and 100 ng/ml [16, 17]. Parikh et al. [18] noted that the optimal cutoff value of uNGAL/Cr to predict delayed graft function after renal transplantation was 1,000 ng/mg. Our results suggest that the optimal cutoff value for predicting UTI is lower than the values determined for AKI. Moreover, it has been previously demonstrated that uNGAL is markedly increased in patient with chronic kidney diseases and reflects disease severity [19, 20]. It is important to note that urine NGAL can potentially be used for early prediction of UTI in children, but only in the absence of AKI and chronic kidney disease.

Another result of our study was that mean uNGAL was found to be higher in patients with renal scar than in those without scar, whereas mean uNGAL/Cr was not different in these two groups. One limitation of this study is that our results regarding acute pyelonephritis and renal scar are not sufficient to interpret due to the limited number of the patients in each group. Moreover, our results should not be extrapolated to children with more subtle presentations, because we examined only symptomatic children. Further investigations are required to determine uNGAL level in patients with pyelonephritis and renal scar. The prevalence of renal scar was 20% in our study, although all patients in the UTI group received early antibiotic treatment. We consider that some of these scars might be attributed to previous UTI, because 58.3% of the patients with scars had a prior history of UTI.

To our knowledge, this is the first study demonstrating that uNGAL is increased in children with UTI and that it might serve as an early predictive biomarker of infection. Our findings suggest that both uNGAL and uNGAL/Cr are excellent indicators for predicting UTI in children, with high sensitivity, specificity, and AUC value in the absence of AKI and chronic kidney disease.

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