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URINE NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (uNGAL) AS PREDICTOR OF RENAL SCARRING

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Urine Neutrophil gelatinase associated lipocalin (uNGAL) is a novel protein expressed in injured epithelia. The aim of our study was to assess whether uNGAL could represent a novel biomarker of renal scarring and to determine the optimal cut-off level for uNGAL to predict the presence of renal scars in children.

Forty-four patients with renal scarring and 44 patients without renal scarring on dimercaptosuccinic acid scan were enrolled in the study. Serum urea and creatinine levels were normal in all children in the study. Urine NGAL was measured by ELISA.

The mean uNGAL level was significantly higher in children with scars than in those without scars (14.92 ng/mL vs 5.71 ng/mL, p=0.001). According to receiver operating curve (ROC) analysis, the optimal cut-off level was 4ng/mL for uNGAL to predict renal scars. Using a cut-off 4ng/mL for uNGAL for prediction of scars, sensitivity and specificity were 68% and 68%, respectively. The positive and negative predictive values of this cut-off point were 68% and 68%, respectively. The area under the curve (AUC) was found to be 0.69 for uNGAL.

In conclusion, the uNGAL level was higher in children with renal scars than in without scars. However, uNGAL has low sensitivity and specificity as a marker for prediction of renal scarring.

Descriptors: URINARY TRACT INFECTIONS; NEUTROPHILS; LIPOCALINS; NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN PROTEIN; CHILD

INTRODUCTION

Renal scarring is a term that describes both congenital and acquired irreversible renal damage which can be associated with the later development of hypertension, proteinuria and chronic renal insufficiency (1, 2). Although the aetiology of renal scarring is still unclear, it has been shown that acquired renal scarring is a sequel of recurrent urinary tract infection (UTI) or vesicoureteral reflux (VUR). Recently dimercaptosuccinic acid (DMSA) radionuclide scan has been the method preferred to detect renal scarring (1, 2). UTI is one of the most common infections in childhood and more than one DMSA scan is performed to determine renal scarring in patients with recurrent UTI during their follow up. In our opinion, a new non-invasive marker for prediction of renal scarring would be useful to diminish the number of DMSA scans.

NGAL is a novel protein identified in human neutrophil granules and has been demonstrated to be expressed in injured epithelium such as renal tubular cells (3, 4). It has been reported that uNGAL is increased in acute kidney injury (AKI) induced by nephrotoxins or ischemia and in chronic kidney disease (CKD) (4-10). Renal scarring may be considered as a form of renal injury. Recently, Ichino et al. (5) constituted experimental pyelonephritis in rats and established that uNGAL in rats increased in both acute pyelonephritis and renal scarring. Therefore, the authors suggested that uNGAL might be used as a diagnostic biomarker for renal scarring.

The aim of our study was to assess whether uNGAL could represent a novel biomarker of renal scarring and to determine the optimal cut-off level for uNGAL to predict the presence of renal scars in children.

PATIENTS AND METHOD

This multicenter, prospective study was conducted between January and June 2009 in Turkey. This study was approved by the local ethics committee and performed according to the ethical standards of the Declaration of Helsinki. Eighty-eight children who suffered recurrent UTI

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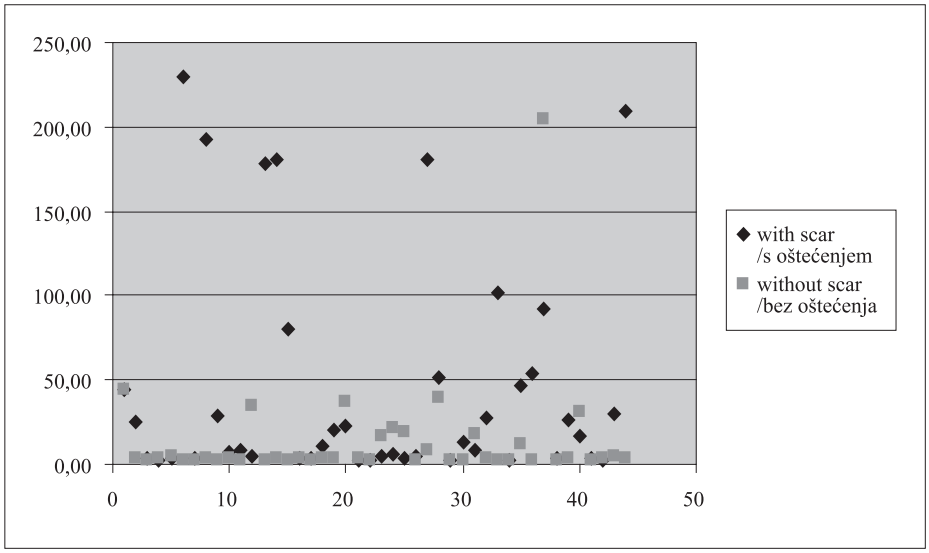

Figure 1. *The distribution of uNGAL levels in the patients with and without renal scars*
Slika 1. *Distribucija razina uNGAL-a u bolesika s bubrežnim oštećenjem i bez njega*

Table 1. *Comparison of uNGAL and uNGAL/creatinine levels by patient groups*
Tablica 1. *Usporedba razina uNGAL-a i uNGAL/kreatinina po skupinama bolesnika s bubrežnim oštećenjem i bez njega*

		Urine NGAL (ng/ml) NGAL u urinu (ng/ml)	p	Urine NGAL/cr (ng/mg) NGAL u urinu/ kreatinin (ng/mg)	p
Patient without scar (S0 group) (n=44) Bolesnici bez bubrežnog oštećenja (S0 skupina) (n=44)		5.71	0.001	14	0.125
Patients with scar (S1 group) (n=44) Bolesnici sa bubrežnim oštećenjem (S1 skupina) (n=44)		14.92		21.51	
With scar group Skupina s bubrežnim oštećenjem	Unilateral scar (n:35) Unilateralno oštećenje (n:35)	15.32	0.909	22.5	0.886
	Bilateral scar (n:9) Bilateralno oštećenje (n:9)	13.45		18.08	
	Volume loss (-) (n:22) Gubitak volumena (-) (n:22)	14.78	0.888	21.97	0.925
	Volume loss (+) (n:22) Gubitak volumena (+) (n:22)	15.05		21.07	
	Function loss (-) (n:8) Gubitak funkcije (-) (n:8)	22.58	0.300	29.16	0.482
	Function loss (+)(n:36) Gubitak funkcije (+) (n:36)	13.60		20.11	

were enrolled in the study after obtaining informed consent from their legal guardians. Fifty-two patients who had been followed up for renal scarring on their prior DMSA scans were invited to hospital, and 44 of them agreed to participate in our study. Physical examination was performed in those 44 patients and a current DMSA scan was performed to evaluate progression of the scars if the patient had not had a scan in the past 6 months. The DMSA scan was performed 2 hours 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 X 128 matrix for a minimum of 500,000 counts each. A scar was defined as a marked localized deformity of the kidney outline with no DMSA uptake on at least two DMSA scans in the absence of UTI (11). The S1 group was composed of the 44 children with renal scars. The S0 group was composed of 44 children who applied to our outpatient clinic consecutively due to recurrent UTI without renal scarring on DMSA scan.

Urine culture, urinalysis, serum urea and creatinine, C-reactive protein (CRP) and serum white blood cell count measurements were performed in both groups at the time of presentation, in order to establish any infection or impaired renal function. Leukocytosis was defined as a leukocyte count more than normal value according to age (12) and positive CRP was defined as CRP value more than 5 mg/L.

Urine samples for culture were obtained by collecting bag or midstream urine in both groups. The children were excluded from the study if they had any bacterial growth in their urine. Urinalysis including leukocyte esterase reaction, nitrite test and microscopic analysis of urine was performed by an Iris IQ 200 full automatic urine analyzer.

Random urine samples were obtained for measurement of uNGAL and creatinine from both groups at the time of presentation. The urine samples were immediately centrifuged at 4 °C for 15 minutes at 13 000 ×g. Aliquots of urine supernatant were stored at – 80 °C for assaying. Urine NGAL was determined using Human NGAL/Lipocalin-2 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 ng/mL.

Statistical calculations were performed with the NCSS 2007 program for Windows. Besides standard descriptive statistical calculations (mean, standard deviation, median and geometric mean), the Kruskal-Wallis test was used in the com-

parison of groups, the post Hoc Dunn's multiple comparison test was utilized in the comparison of subgroups, the Mann-Whitney-U test was used in the comparison of the two groups, and the Chi square test was performed during the evaluation of qualitative data. The results were evaluated to within a 95% confidence interval. 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 a geometric mean uNGAL and uNGAL/Cr. ROC analysis was performed to determine sensitivity and specificity of different cut-off points for uNGAL and uNGAL/Cr for the prediction of renal scarring. The optimal cut-off point was chosen according to ROC analysis and the AUC was calculated. Statistical significance level was established at $p<0.05$.

RESULTS

The S1 group consisted of 44 children (30 female, 14 male) and the mean age was 9.17±3.89 years (range: 3-18 years). The S0 group consisted of 44 children (33 females, 11 males) and mean age was 7.85±3.73 years (0.78-15 years). Serum urea and creatinine levels were normal in all children with and without renal scars. None of the patients had infection at the time of the study.

The mean uNGAL level was significantly higher in the S1 group than in S0 (14.92 ng/mL vs 5.71 ng/mL, p=0.001) (figure 1, table). According to ROC analysis, the optimal cut-off level was 4ng/mL for uNGAL to predict renal scars. Using a cut-off 4ng/mL for uNGAL for prediction of scars, sensitivity and specificity were 68% and 68%, respectively. The positive and negative predictive values of this cut-off point were 68% and 68%, respectively. When uNGAL is higher than this cut-off value, the possibility of the presence of renal scars increases twice (positive likelihood ratio: 2.14 and negative likelihood ratio: 0.47). AUC was found 0.69 for uNGAL (figure 2).

Mean uNGAL/Cr ratio was not significantly different in the patients with or without scars (21.51 ng/mgCr vs 14 ng/mgCr; p=0.125) (table). Mean uNGAL and uNGAL/Cr ratio were not related to the presence of unilateral or bilateral scars, volume loss of kidney or function loss of kidney (p>0.0.5) (table 1).

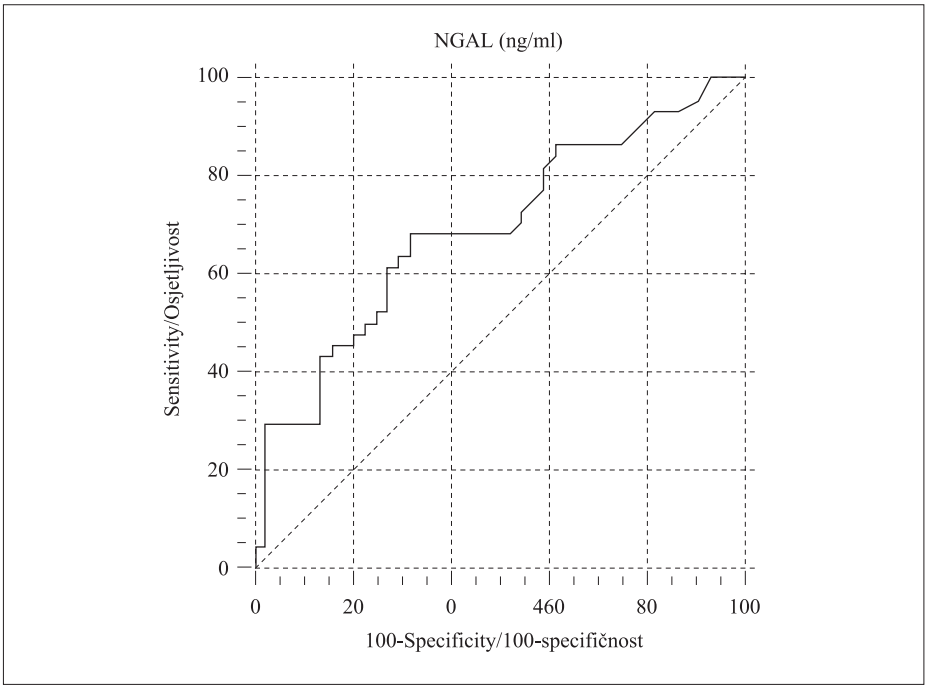

Figure 2. *Receiver operating curve to predict renal scars (AUC = 0.69)*
Slika 2. *ROC analiza, površina ispod krivulje (AUC = 0.69)*

DISCUSSION

NGAL, a protein that is originally derived from human neutrophils, is expressed in injured epithelia, such as renal tubular cells (3, 4). Recent studies have demonstrated that uNGAL is increased in AKI induced by nephrotoxins or ischemia and in CKD (4-10, 13). Moreover, it has been considered that NGAL expression is induced in order to contribute to tissue regeneration after kidney damage (5).

Renal scarring is a kind of renal damage which varies in localized hypoactive areas in kidneys on DMSA scan according to volume and function loss of the kidney. It might be possible that uNGAL, as a marker of renal injury, may be used as a predictor of renal scaring. Supporting this idea, increased levels of uNGAL have been demonstrated in a rat model of renal scarring (5). In this rat model, *Escherichia coli* was injected into the renal cortex to constitute an experimental pyelonephritis and renal scarring developed 6 weeks after injection (5). It was observed that uNGAL levels peaked within one week following injection of *Escherichia coli* into the renal cortex (5). Although uNGAL decreased to a lower level in the second week, it remained stable until renal scarring developed in the 6th week and never decreased to normal levels (5). As a result, the uNGAL level was found to be higher than in controls at the time of renal scarring and the authors concluded that uNGAL may be a diagnostic marker of renal scars (5). Therefore, we aimed to assess whether increased uNGAL level may represent a novel, sensitive marker of renal scarring in children. The urine test was preferred because obtaining urine is easy and painless for the child. In our study, we established that uNGAL was also higher in children with renal scars than in those without scars, and determined the optimal cut-off level for uNGAL to predict renal scars in children with normal serum creatinine. We also found that there was no significant relationship between uNGAL levels and the severity of renal scarring.

According to our results, the optimal cut-off point for prediction of renal scars was 4 ng/mL and was much lower than the values determined for AKI. Hirsch et al. (6) reported that AKI due to contrast administration can be predicted using a cut-off of 100 ng/mL for uNGAL. In different studies, the cut-off values for prediction of AKI after cardiopulmonary bypass were determined as 50 ng/mL and 100 ng/mL (7, 8). Parikh et al. (9) reported that the optimal cut-off value of uNGAL/Cr to predict delayed graft function after renal transplantation was 1000 ng/mgCr. Moreover, we had previously demonstrated that both uNGAL and uNGAL/Cr can be used as sensitive markers

for early prediction of UTI in children using a cut-off of 20 ng/mL and 30 ng/mgCr, respectively (14). The sensitivity and specificity of uNGAL for prediction of UTI were 97% and 76%, respectively. However, the optimal cut-off point of uNGAL for prediction of renal scars is very low and sensitivity and specificity were lower at this cut-off level. We consider that uNGAL seems to be a more valuable marker in prediction of UTI than renal scars and should be used to predict UTI, although a lower level of uNGAL was also predictive for renal scarring. Our idea was supported by the results that peak uNGAL level was seen in the early stages of infection rather than at the time of development of renal scars in the rat model of Ichino et al. (5).

Another result of the present study was that uNGAL/Cr was not significantly different in patients with or without scars. In our previous study investigating the value of uNGAL for prediction of UTI, mean uNGAL had been found to be higher in the patients with renal scars than in those without scars, whereas mean uNGAL/Cr had not been different in these two groups, supporting the results of the present study. The major drawback of our previous study was the small number of patients with renal scars (n:12). In our present study, we confirmed the results of our previous study in a larger group of children.

To our knowledge, this is the first study demonstrating that uNGAL increased in children with renal scars and evaluating the optimal cut-off level to predict renal scarring. Although our results revealed that uNGAL was higher in the patients with renal scars than in those without scars, the sensitivity and specificity of uNGAL for prediction of renal scars was lower than for prediction of UTI.

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Sažetak

NEUTROFIL ŽELATINAZA UDRUŽENI LIPOKALIN U URINU (uNGAL) KAO PREDIKTOR BUBREŽNOG OŠTEĆENJA

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Neutrofil želatinaza udruženi lipokalin u urinu (uNGAL) novi je protein čija se ekspresija povećava kod oštećenja epitelnih stanica. Cilj ove studije bio je odrediti može li uNGAL biti novi biomarker bubrežnog oštećenja i utvrditi kritičnu razinu uNGAL-a koja bi upućivala na njegovo postojanje u djece.

U ispitivanje je bilo uključeno 44-ero bolesnika s bubrežnim oštećenjem i 44-ero bolesnika bez bubrežnog oštećenja, koje je potvrđeno studijom s dimerkaptosukciničnom kiselinom. Razine ureje i kreatinina u serumu bile su u granicama normale kod svih ispitanika. Razina uNGAL-a mjerena je ELISA-om.

Srednja vrijednost razine uNGAL-a bila je statistički značajno viša u djece s bubrežnim oštećenjem u odnosu na djecu bez njega (14,92 ng/mL vs 5,71 ng/mL, p=0,001). Temeljem podataka dobivenih ROC analizom optimalna kritična razina uNGAL-a koja bi upućivala na bubrežno oštećenje bila je 4 ng/mL. Uzimajući razinu uNGAL-a od 4 ng/mL kao kritičnu razinu za predviđanje bubrežnog oštećenja, osjetljivost testa bila je 68%, a specifičnost 68%. Pozitivne i negativne prediktivne vrijednosti ove kritične razine bile su 68% i 68%. Površina ispod krivulje za uNGAL bila je 0,69.

Zaključno, razina uNGAL-a bila je viša u djece s oštećenjem bubrega u odnosu na djecu bez njega. No mjerenje uNGAL-a kao prediktora bubrežnog oštećenja ima nisku osjetljivost i specifičnost.

Deskriptori: INFEKCIJE URINARNOG TRAKTA; NEUTROFILI; LIPOKALIN; NEUTROFIL ŽELATINAZA UDRUŽENI LIPOKALIN U URINU; DIJETE

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