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Original Article

Urinary neutrophil glatinase-associated lipocalin level (uNGAL) may predict the severity of congenital hydronephrosis in infants

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Abstract: Clinical findings suggest that the urinary neutrophil gelatinase-associated lipocalin (uNGAL) level may be a highly sensitive biomarker and predictor of progressive tubular and glomerular injury. This cross-sectional study aimed to determine the predictive power of uNGAL in infants with congenital hydronephrosis. Forty-five children (30 males and 15 females) under the age of two with congenital obstructive uropathy were evaluated for urinary levels of creatinine, uNGAL and uNGAL/uCreatinine (Cr) ratio. Totally, 62.2% of patients had mild, 15.6% had moderate and 22.2% had severe hydronephrosis. We observed a higher significantly uNGAL level in cases with severe form than cases with mild to moderate forms (P=0.002). Also, infants with severe hydronephrosis showed a higher ratio of uNGAL/uCr compared with mild to moderate cases (P=0.006). Correlation analysis showed a significant inverse correlation between uCr levels and pelvic diameter (P=0.002) and direct correlations between uNGAL and uNGAL/uCr ratio and pelvic diameter (P<0.001). By defining a cut-off point of 73.7 ng/ml for uNGAL in ROC analysis, we observed a sensitivity of 70.0% and a specificity of 91.4% forthe prediction of severe hydronephrosis. Our results indicate the potential predictive valueof uNGAL and uNGAL/uCr ratio for hydronephrosis and, more importantly, for discrimination of the severe hydronephrosis from mild to moderate forms.

Keywords: Congenital hydronephrosis, urinary, neutrophil gelatinase-associated lipocalin

Introduction

One of the most important causes of chronic kidney disease in children is obstructive nephropathy, which is a secondary consequence of congenital obstructive uropathiesand requires therapeutic interventions to reduce the risk of permanent renal injuries [1-4]. This congenital disorder, known as hydronephrosis, is often non-obstructive and resolved spontaneously in 70% of cases within the first few weeks of life [5]. Hydronephrosis is characterized by demonstrating a dilated renal pelvis and/or calyces [6-8]. When the fetus is suspected of having a genitourinary tract abnormality, the main concerns are differential diagnosis, assessing the associated anomalies and determining the pre- and post-natal risks of anomalies [5].

Diagnosis of antenatal hydronephrosis is carried out by several approaches, including serial renal sonograms, a voiding cystourethrogram (VCUG) and a diuretic renogram or excretory urogram (intravenous pyelography), which provide the necessary diagnostic information for better management of this congenital disorder [4]. Although some studies have identified the antenatal hydronephrosis as a benign disorder, others believe that this disease may lead to the permanent kidney damage. Therefore, earlier management would be more helpful to prevent more renal injuries [9-11]. There is currently no definitive predictive marker for assesingto evaluate the clinical outcomes of congenital hydronephrosis (e.g. the severity of obstruction and the subsequent renal injuries), and the current approach for managingthis disorder is postnatal ultrasound, which is an invasive, inaccu-

Table 1. The basic and clinical characteristics of the study patients (n=45)

Variables	Numbers (%)
Gender	
Male	30 (66.7%)
Female	15 (33.3%)
Age in month (Mean ± SD)	8.9±11.6
Hydronephrosis Severity	
Mild	28 (62.2%)
Moderate	7 (15.6%)
Severe	10 (22.2%)
uCr (mg/dl)	53.3±39.6
uNGAL (ng/ml)	80.43±70.92 (MED=50)
uNGAL/uCr Ratio (Mean ± SD)	3.71±5.29 (MED=1.43)

uNGAL: urinary neutrophil gelatinase-associated lipocalin, uCr: urinary creatinine, MED: median.

rate and a subjective method during the first weeks of neonatal age [5, 7].

Due to the limitations of ultrasound method to determine the severity of obstruction and tissue damage, the search fora more reliable marker for the evaluation of renal injuries in order to make the right decision, including surgical interventions or conservative treatments, is still an active area of research.

Nowadays, measuring urinary biomarkers as a non-invasive method is one of the promising approaches that can be widely used to diagnose and to manage kidney disorders [12-14]. In this context, neutrophil gelatinize associated lipocalin (NGAL) as a 25 KDa protein, which is produced by damaged nephrons epithelium, is one of the newest biomarkers for kidney damages. Unlike measuring the serum creatinine level and urinary output to evaluate kidney function, urinary NGAL (uNGAL) which is specifically produced by damaged nephrons and released into the blood and urine, could be a more accurate and promising indicator of kidney injury [13-15]. The results of a pilot study clearly demonstrated that the increased levels of urinary KIM-1 and NGAL in children with obstructive uropathy were negatively correlated with differential renal function in the radionuclide scan [16].

With this in mind, the present study was designed to explore the relationship between Ungal levels and severity of obstruction and kidney parenchymal damage as well as to determine the ability of uNGAL to predict congenital obstructive uropathy in infants under two years of age.

Patients and methods

Study subjects

In this cross-sectional study, 45 infants under 2 years of age and with congenital obstructive uropathy who referred to our Nephrology Clinic in the Hamadan University of Medical Sciences Hospital between May 2017 and September 2018 were studied. This study was approved by our Institutional Research Ethics Committee (IR.UMSHA.REC.1396.726) and all parents provided the written and signed informed consent before enrollment of their neonates into the study. Neonates with an antenatal history of hydronephrosis confirmed by postnatal ultrasonography were included. Also, neonates with vesicoureteral reflux (VUR) determined by Voiding Cystourethrography (VCUG), metabolic disorder and recent surgical manipulations of the kidneys, ureters and bladder were excluded from the study.

Clinical imaging and diagnosis of hydronephrosis

Renal ultrasonography was used to assess the cortical thickness and anterior-posterior diameter of renal pelvis as an initial diagnostic tools for detection of hydronephrosis. The severity of hydronephrosis was defined based on anterior-posterior diameter of renal pelvis as follows: 5 mm of diameter was considered as normal, 5-9 mm as mild, 10-15 mm as moderate and more than 15 mm was classified as severe hydronephrosis [17, 18].

Then, neonates with persistent significant hydronephrosis (pelvic diameter >15 mm) were examined by dynamic renal scan using 99 mTc-DTPA to confirm the presence of any obstruction and the severity of renal dysfunction. Obstruction was defined based on the significant increase in half-clearance time ($T_{1/2}$>15 minutes) of tracer uptake.

Laboratory measurements

After diagnosis of prenatal hydronephrosis, 10 ml of urine sample was collected for measuring uNGAL and uCr levels. uNGAL levels were mea-

Table 2. Comparisons of the mean urinary creatinine and NGAL levels as well as the mean uNGAL/uCr ratios between patients' subgroups according to the diseases severity

Biomarkers	Mild (n=28)	Moderate (n=7)	Severe (n=10)	*P Value*
uCr (mg/dl)	62.50±38.35	51.50±48.10	28.80±27.87	*0.66**
uNGAL (ng/ml)	52.96±20.42	81.28±57.53	156.86±110.86	*0.002***
uNGAL/uCr ratio	1.75±2.28	3.34±4.24	9.46±7.76	*0.006***

Note: Analyses were done by One-way ANOVA* and Kruskal Wallis** with post Tukey test. Post-test analysis revealed significant differences for uNGAL levels and uNGAL/uCr ratios between the severe form and mild as well as moderate forms but not between the mild and moderate forms. uCr: urinary creatinine, uNGAL: urinary neutrophil gelatinase-associated lipocalin.

sured using a quantitative Enzyme-Linked Immunosorbent Assay (ELISA) kits (Human NGAL Lipocalin-2/NGAL ELISA kit; Bio Vendor Diagnostic, Brno, Czech Republic) as per manufacturer's instructions. Also, uCrlevels (mg/dl) were quantified by CREA Creatinine Jaffe method compensated (Roche Diagnostics, Mannheim, Germany). In addition, the ratios of uNGAL/uCr levels were calculated for all samples. Finally, urinary levels of biomarkers as well as uNGAL/uCr ratios were compared between the patients subgroups based on the severity of hydronephrosis and anterior-posterior diameters of renal pelvis.

Finally, sensitivities and specificities of uNGAL and uNGAL/uCr ratio for prediction of the severity of obstructive uropathy were calculated by drawing ROC curve at a cutoff point of 73.7 ng/ml for uNGAL and 3.87 for uNGAL/uCr ratio.

Statistical analysis

Comparison of the mean levels of uNGAL, creatinine and the mean uNGAL/uCr ratio between the patients' subgroups were performed by *t-test* and one-way analysis of variance as well as Kruskal-Wallis and Mann-Whitney tests when appropriate. The sensitivities and specificities of uNGAL and uNGAL/uCr ratio for the prediction of uretero-pelvic junction obstruction were calculated using ROC curve analysis and comparison of the area under curve (AUC) for each biomarker. The best cutoff points for both variables were chosen when the sum of sensitivity and specificity had the largest values. A *P*-value of <0.05 was considered statistically significant. All statistical analysis were performed by SPSS ver. 21 (SPSS Inc., Chicago, IL, USA).

Results

Forty-five infants with congenital obstructive uropathy (30 males and 15 females) and with the mean age of 8.93±11.6 months (ranged between 1-24 months) were analyzed in this cross-sectional study. Based on the obstruction severity and the presence of renal dysfunction, our patients were divided into three groups, including group I: mild (28 out of 45 cases), group II: moderate (7 out of 45 cases) and group III: severe hydronephrosis (10 out of 45 cases) (**Table 1**).

The mean levels of uCr and uNGAL were 53.30±39.59 mg/dl and 80.43±70.92 ng/ml respectively. Also, the mean $\frac{urinary\ NGAL}{urine\ creatinine}$ ratio was 3.71±5.29 in all studied infants. Comparison of the mean levels of uNGAL between three subgroups of the patients revealed a higher significantly uNGAL levels in cases with severe form of disease than mild to moderate forms (P=0.002, **Table 2**). Similarly, infants with severe hydronephorsis showed higher ratio of uNGAL/uCr compared with mild tomoderate cases (P=0.006, **Table 2**). Post-Hoc analysis by Tukey test also showed significantly differences for uNGAL levels between severe and mild (P<0.001) or moderate (P= 0.03) forms as well as for uNGAL/uCr ratio between severe cases and mild (P=0.001) or moderate (P=0.001) forms.

Spearman rank correlation analysis showed a significant inverse correlation between uCr levels and pelvic diameter (P=0.002) and direct correlations of uNGAL and uNGAL/uCr ratio with pelvic diameter (P<0.001, **Figure 1**).

The sensitivities and specificities of uNGAL and uNGAL/uCr ratio for prediction of hydronephrosis severity were calculated by ROC analysis. Considering the quantitative data in ROC analysis to discriminate the subgroups of patients revealed that the definition of a cut-off point of 73.7 ng/ml of uNGAL has 70.0% sensitivity and 91.4% specificity (P=0.001) for the prediction

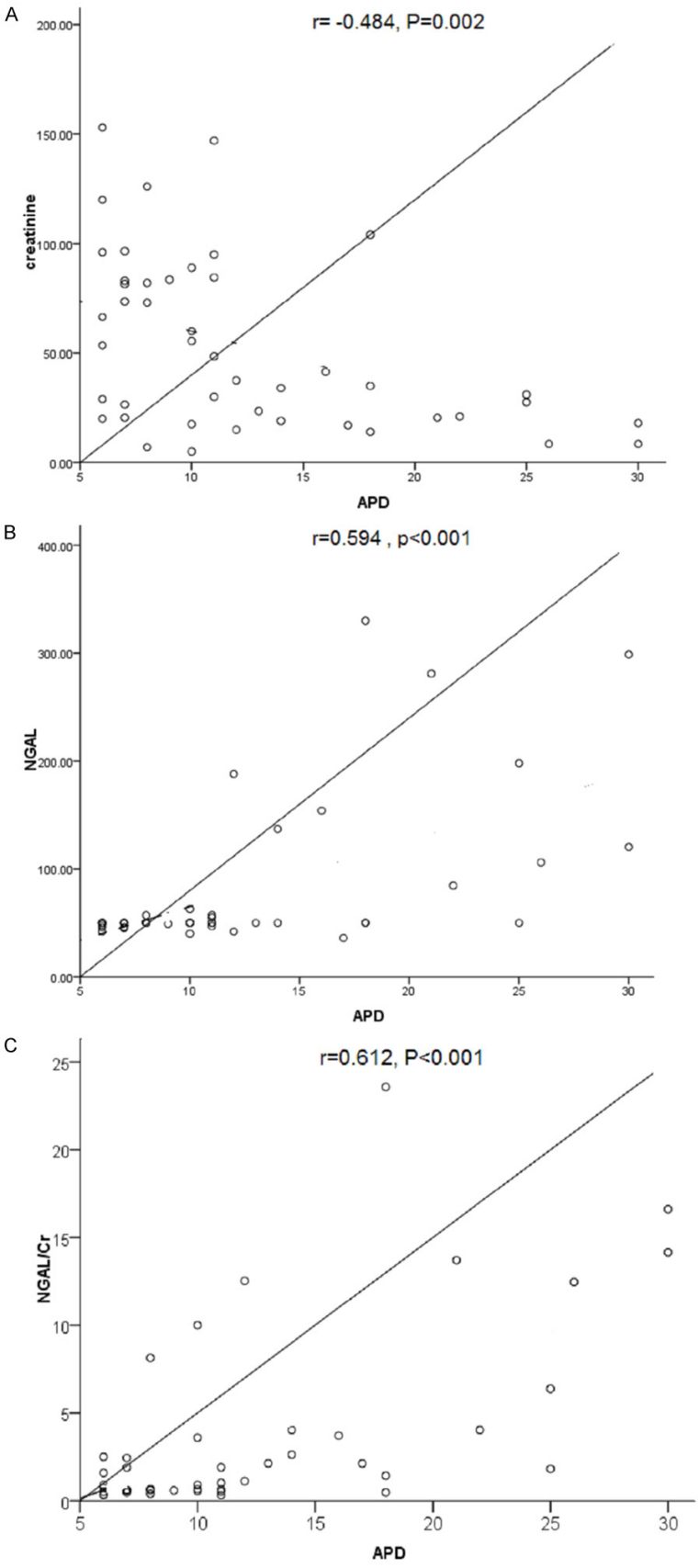

Figure 1. Spearman rank correlation analysis between urinary creatinine, urinary NGAL and urinary NGAL/Cr ratio and anterior-posterior pelvic diameter (APD). NGAL: neutrophil gelatinase-associated lipocalin.

of severe hydronephrosis. Also, considering of cutoff point of 3.87 for uNGAL/uCr ratio showed 70.0% sensitivity and 88.6% specificity (P= 0.003) for predicting severe hydronephrosis (**Figure 2**).

Discussion

Future challenges for the evaluation and treatment of patients with obstructive uropathy are related to the indication for surgical intervention and finding prognostic indicators for the progression of renal injuries. In the clinical setting, there is no a clear indication for the timing of surgical intervention to prevent kidney damage [5-8].

In this situation, although diuretic renograms and ultrasound evaluation may assist the clinicians to diagnose the presence and severity of anobstructive uropathy, there is an urgent need to look for the potential biomarkers, preferentially urinary markers that allows the early detection of obstructive uropathy and deterioration in renal function [4, 7]. The uNGAL as a potential early biomarker of tubular injury can indicate the extent of subclinical injures, especially in obstructive disorders that begin in the first weeks of life [16].

In the present study, we evaluated the predictive power of uNGAL level and uNGAL/uCr

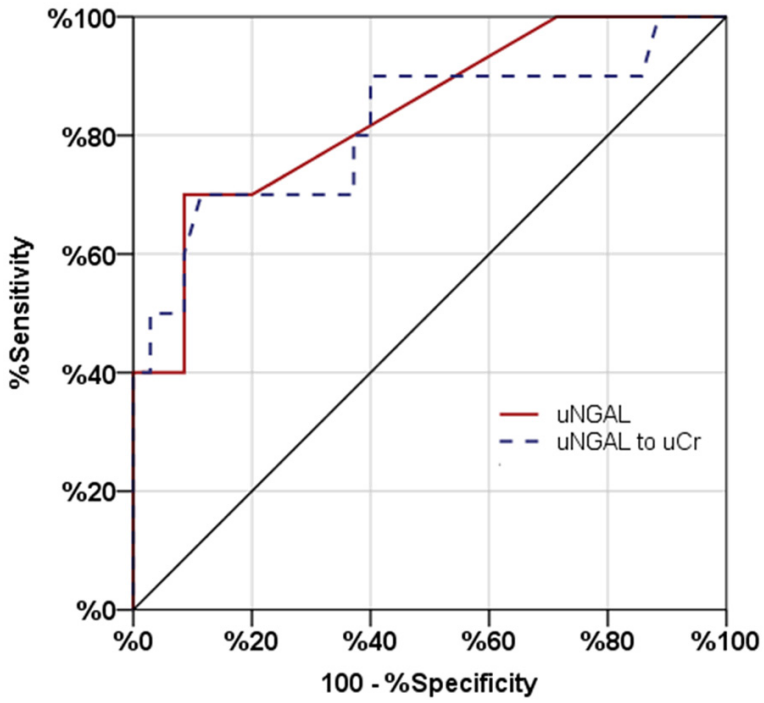

Figure 2. Receiver operating characteristic (ROC) curveanalysis for determining the sensitivities and specificities of uNGAL and uNGAL/uCr ratio to predict the severe hydronephrosis. uNGAL: urinary neutrophil gelatinase-associated lipocalin, uCr: urinary creatinine.

ratio to discriminate between different grades of antenatal hydronephrosis. We observed the significantly increased levels of uNGAL and uNGAL/uCr ratio in the infants with severe hydronephrosis compared with mild to moderate forms. Moreover, we found the direct correlations of uNGLA and uNGAL/uCr ratios with kidney pelvic diameter in all patients regardless of the severity of obstruction. By defining a cutoff point of 73.7 ng/ml for uNGAL, and 3.87 for uNGAL/uCr ratio we determined a sensitivity of 70.0% and specificity of 91.4% for uNGAL as well as 70.0% sensitivity and 88.6% specificity for uNGAL/uCr ratio to discriminate the severe hydronephrosis from the mild form of this disease in infants.

Our findings are line with kostic *et al.* [19] and Madsen *et al.* [20] studies that showed the increased levels of urinary NGAL in patients suffering from hydronephrosis. Similarly, Wasilewski *et al.* [16] and Almodhen *et al.* [21] studies demonstrated a meaningful statistical correlation between the severity of obstruction and uNGAL levels. Consistently, we found a meaningful and direct correlations between kidney pelvic diameter and uNGAL level and uNGAL/uCr ratio, which is in agreement with Cost et al. study of demonstrating a significant correlation between urinary NGAL and renal function [22].

Alternately, a significant increases of the NGAL protein synthesis in the dilated thick ascending limb of Henle has also been shown in a mouse model of unilateral obstruction [23].

Our results with regard to the sensitivity and specificity of uNGAL for prediction of renal injury can also be supported by Ma *et al.* study [24] that showed the higher significantly urinary NGAL levels in the hydronephrotic kidneys than normal kidneys in a group children. They also reported a significant direct correlation between uNGAL levels and histopathologic grades in the hydronephrotic kidneys. Likewise, Skalova *et al.* study of 31 children with hydronephrosis demonstrated a significantly higher urinary NAG/Cr ratio in the patients with hydronephrosis compared with reference data [25]. As expected, we found a meaningful relationship between the severity of obstruction and uNGAL level as well as uNGAL/uCr ratio in children with obstructive uropathy.

Similarly, Seo et al. study showed a sensitivity of 75% and specificity of 83.3% for this biomarker in children with urinary tract infection [26]. Also, Kim et al. study of children with acute pyelonephritis demonstrated that plasma levels of NGAL is comparable with CRP, procalcitonin and WBC to predict acute pyelonephritis [27]. In accordance with these findings, our previous report [12] and Nikavar et al. [28] study showed the significantly increased levels of uNGAL and uNGAL/uCr ratio in children with vesicoureteral reflux.

Altogether, despite a major limitation in the present study (small number of patients), our

results indicate a potential predictive value of uNGAL and uNGAL/uCr ratio for hydronephrosis and, more importantly, for discrimination of the severe hydronephrosis from the mild to moderate forms of congenital hydronephrosis. However, our findings should be interpreted with caution, and certainly more studies using larger cohorts of the patients are warranted to find out the exact relationship between uNGAL and the severity of prenatal hydronephrosis and to validate the predictive/prognostic value of this urinary biomarker in patients with obstructive uropathy.

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Disclosure of conflict of interest

None.

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