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Urinary NGAL Levels Correlate with Differential Renal Function in Patients with Ureteropelvic Junction Obstruction Undergoing Pyeloplasty

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Abstract

Purpose: Recent investigations described the use of NGAL, a sensitive biomarker for kidney injury, in the setting of ureteropelvic junction obstruction. We prospectively evaluated urinary NGAL levels in the affected renal pelvis and bladder of children with ureteropelvic junction obstruction undergoing unilateral dismembered pyeloplasty. Our hypothesis was that higher NGAL in the kidney and bladder would correlate with decreased ipsilateral differential function.

Materials and Methods: We performed a prospective cohort study in patients treated with unilateral dismembered pyeloplasty from 2010 to 2012. Urine was obtained intraoperatively from the bladder and obstructed renal pelvis. A control population of unaffected children was recruited to provide a voided bladder specimen. Bladder NGAL levels were compared between the study and control populations. We tested our study hypothesis by correlating bladder and renal pelvic NGAL levels with the differential renal function of the affected kidney.

Results: A total of 61 patients with a median age at surgery of 1.62 years (range 0.12 to 18.7) were enrolled in the study. Median bladder NGAL was 18.6 ng/mg (range 1.4-1,650.8) and median renal pelvic NGAL was 26.2 ng/mg (range 1.2-18,034.5, $p = 0.004$). Median bladder NGAL was significantly higher than in controls ($p = 0.004$). The correlation of bladder and renal pelvic NGAL with differential renal function was $r = -0.359$ ($p = 0.004$) and $r = -0.383$ ($p = 0.002$), respectively.

Conclusions: Bladder NGAL is increased in children with ureteropelvic junction obstruction. Renal pelvic and bladder normalized urinary NGAL levels correlate inversely with the relative function of the affected kidney in cases of unilateral ureteropelvic junction obstruction.

Keywords

urinary bladder; kidney; ureteral obstruction; LCN2 protein, human; biological markers

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Hydronephrosis is one of the most common prenatally detected abnormalities of the urinary tract, found in 1% to 5% of pregnancies, and significant UPJO is discovered in 19% to 25% of these patients.[1] Since the advent of widespread prenatal ultrasound, many infants with UPJO have been diagnosed long before they become symptomatic with flank pain, gross hematuria and/or urinary tract infections. For many years there has been controversy regarding the indications for surgical intervention in asymptomatic patients. The concern is that children with benign and self-limiting urinary tract dilatation may be overtreated. Some investigators proposed initial nonoperative management along with intensive imaging protocols with surgical intervention primarily based on decreasing ipsilateral differential renal function or increasing drainage $T_{1/2}$.[2,3]

However, in the era of personalized medicine urinary biomarkers have been proposed for diagnosing and managing UPJO in children as a noninvasive adjunct to radiographic evaluation to assist with decision making.[4-6] Urinary NGAL is an early, sensitive biomarker for kidney injury that was analyzed in the setting of obstructive uropathy.[7-9] In the setting of UPJO a recent observational study described increased NGAL in the renal pelvic urinary aspirates of patients undergoing pyeloplasty compared to controls.[10] However, these data were not correlated with any clinical end points.

We evaluated uNGAL levels in the renal pelvis and bladder in children treated with dismembered pyeloplasty and correlated this information with clinical data on renal function. Our general aim was to evaluate the potential of uNGAL as a noninvasive clinical biomarker of renal injury in the setting of UPJO.

MATERIALS AND METHODS

Populations

Study—We performed a prospective cohort study in patients with unilateral UPJO treated with laparoscopic or open dismembered pyeloplasty at a single pediatric institution from 2010 to 2012. Study exclusion criteria included prior ipsilateral renal surgery, contralateral untreated UPJO, ipsilateral ureteral dilation or high grade vesicoureteral reflux. Institutional review board approval was obtained for the study and written informed consent was obtained in all cases.

Control—A group of healthy, nonaffected children younger than 18 years was recruited to provide voided bladder urine specimens for NGAL analysis. They were prescreened by history and urinalysis to exclude any with preexisting urological or nephrological issues.

Study

Design—Patient demographics, radiographic imaging, intraoperative details and surgical outcomes were abstracted from the medical record. Urine samples were obtained intraoperatively from the bladder by cystoscopy or urethral catheterization. The obstructed renal pelvis sample was needle aspirated from the pelvis intraoperatively immediately before UPJO transection or drawn from a ureteral catheter at retrograde pyelogram. Samples were immediately stored in a laboratory refrigerator and batch analyzed for NGAL using the NGAL Test™ Reagent Kit RUO enzyme-linked immunosorbent assay.[11] Samples were

refrigerated not more than 24 hours before centrifugation for 15 minutes at 4C. Samples were aliquoted, immediately frozen at −80C and stored at −80C for no more than 3 months. The different lots of assays underwent strict quality control at the manufacturer with an interassay variation of well below 5%. Urinary creatinine was measured by a modified Jaffe assay using a Dimension® Xpand® Plus HM analyzer (coefficient of variation 2.5% to 3.0%) and standardized to the international standard to account for urine dilution/hydration status.

Objectives—Recently published data show that NGAL is increased in the affected renal pelvic urine sample in the setting of hydronephrosis prompting surgical correction.[10] Thus, our primary study objective was to correlate renal pelvis and bladder NGAL, and the renal pelvis-to-bladder NGAL ratio with ipsilateral differential renal function on preoperative nuclear renography. We hypothesized that renal pelvis uNGAL would be higher than bladder uNGAL and a higher renal pelvis-to-bladder uNGAL ratio would correlate with differential function. Specifically, higher uNGAL in the kidney and bladder as well as the ratio should correlate with decreased ipsilateral differential function. Our secondary study objective was to compare bladder uNGAL levels between unaffected children and those undergoing pyeloplasty for UPJO to validate our study rationale.

Statistical Analysis

Collected data were analyzed with nonparametric methods using SPSS®, version 20.0. Independent continuous variables were compared using the Mann-Whitney U test and independent categorical variables were compared using the chi-square or Fisher exact test. Normalized uNGAL from the renal pelvis and bladder in each patient was compared using the Wilcoxon paired sample signed rank test. A ratio was calculated between uNGAL from renal pelvis and bladder specimens. This ratio, and bladder and renal pelvis uNGAL were then correlated with clinical data using the Spearman ρ correlation coefficient, a nonparametric analysis of bivariate correlation. For all tests statistical significance was considered at $p \leq 0.05$.

RESULTS

A total of 36 males and 25 females were enrolled in the study and had bladder and renal pelvic aspirates sent for NGAL measurement at dismembered pyeloplasty. Median age at surgery was 1.62 years (range 0.12-18.7, 95% CI 3.2-5.8, table 1). All patients had unilateral UPJO at surgery, which was on the right side in 31 and the left side in 30. Of the patients 47 (77.1%) had only intrinsic obstruction, while a crossing vessel was observed in 14 (22.9%). A total of 31 patients (50.8%) presented with symptoms, including urinary tract infection in 16 (26.2%), colic in 12 (19.7%), and poor feeding, cyclical vomiting and abdominal distention in 1 each (1.6%).

Median renal pelvis uNGAL was statistically significantly higher than median bladder uNGAL ($p = 0.004$, table 1). Patients had significantly higher median bladder uNGAL than the 22 controls (table 1). The correlation between uNGAL in patients and differential function was $r = -0.359$ for bladder uNGAL ($p = 0.004$), $r = -0.383$ for renal pelvis uNGAL ($p = 0.002$) and $r = -0.166$ for the uNGAL ratio ($p = 0.2$, table 2). As a reference, the

correlation between $T_{1/2}$ and differential renal function was r = −0.363 (p = 0.007). We observed no significant correlation of age or gender with uNGAL.

DISCUSSION

Hydronephrosis is one of the most common abnormalities found on prenatal ultrasound.[1] It is often a benign, transient process remaining from renal embryological development. In many children it resolves spontaneously with no long-term sequelae. However, persistent UPJO may be present, which may cause progressive harm to the kidney with deterioration of function. The current algorithm for distinguishing between UPJO that requires surgical intervention vs UPJO for which watchful waiting is appropriate includes repetitive invasive radiographic imaging with the crux of the decision making centered on the results of diuretic renogram. Evidence-based guidelines are lacking but generally concern for the health of the kidney is raised when split differential function shows a progressive decrease with or without a concomitant poor washout curve and prolonged $T_{1/2}$ drainage. Unfortunately, this test relies on arbitrary threshold values that may not be reproducible among institutions and may not correlate with biological outcomes.

Recently, there has been interest in urinary biomarkers as an adjunct to radiographic evaluation. Such biomarkers could theoretically help identify a kidney at risk and provide the clinician with more substantial data before proceeding with surgical intervention. Various urinary biomarkers and urinary proteome analysis were proposed in children for congenital obstructive uropathy[9,12] and unilateral UPJO.[13-16] Kajbafzadeh et al evaluated the predictive role of urinary carbohydrate antigen 19-9 in UPJO diagnosis and followup.[15] They found significant increases in a group of children with UPJO compared to normal controls. Followup assessments showed significantly lower levels in the bladder after surgery. Sager et al assessed the role of transforming growth factor-•1, a cytokine detectable in urine in normal conditions.[16] In their study of 19 patients and age matched controls mean renal pelvic levels were higher than vesical levels in patients with UPJO. Bartoli et al studied epidermal growth factor, monocyte chemotactic protein-1 and β2-microglobulin.[13] They compared bladder levels in children with hydronephrosis who had obstructed washout curves, defined as $T_{1/2}$ greater than 20 minutes, on furosemide renography vs those with a nonobstructed curve, defined as $T_{1/2}$ less than 20 minutes. Urinary β2-microglobulin and monocyte chemotactic protein-1 increased significantly in the obstructed group, while epidermal growth factor excretion decreased.

NGAL is a 25 kDa protein belonging to the lipocalin family. uNGAL was described as a promising early marker of acute kidney injury.[17] It has been investigated extensively in experimental and clinical studies of cardiac disease, critically ill patients with septic shock, intravenous contrast nephropathy and severely premature infants.[7,18-21] Its use in congenital UPJO cases may provide a sensitive index for detecting early kidney damage.

The genesis and sources of plasma and urinary NGAL after kidney injury require further clarification. Although plasma NGAL is freely filtered by the glomerulus, it is largely reabsorbed in the proximal tubules.[9,22] Direct evidence for this notion is derived from systemic injection of labeled NGAL, which becomes enriched in the proximal tubule but

does not appear in urine. Thus, any urinary excretion of NGAL is likely only when there is concomitant proximal renal tubular injury that precludes NGAL reabsorption and/or increases de novo NGAL synthesis.

Gene expression studies in kidney injury models demonstrated rapid, massive up-regulation of NGAL mRNA in distal nephron segments, specifically in the thick ascending limb of the loop of Henle and the collecting ducts.[23] The resultant synthesis of NGAL protein in the distal nephron and secretion in urine comprises the major fraction of uNGAL. Since uNGAL is highly protease resistant, it is stable for many hours at room temperature, stable for several days at −20C and stable for 6 to 12 months at −80C.[23]

With respect to plasma NGAL, the injured kidney appears to be only one of the major sources. In animal studies direct ipsilateral renal vein sampling after ischemia indicated that NGAL synthesized in the kidney is not introduced efficiently into the circulation but is abundantly present in the ipsilateral ureter. However, it is now well known that kidney injury results in organ cross-talk and dramatically increased NGAL mRNA expression in distant organs, especially the liver and lungs, and over expressed NGAL protein released into the circulation may represent a distinct systemic pool.

Additional contributions to the systemic pool of NGAL in kidney injury cases may derive from the fact that NGAL is an acute phase reactant that may be released from neutrophils, macrophages and other immune cells. Furthermore, any decrease in the glomerular filtration rate resulting from kidney injury would be expected to decrease NGAL renal clearance with subsequent accumulation in the systemic circulation. The relative contribution of these mechanisms to the increase in plasma NGAL after kidney injury remains to be determined.[23] Therefore, urinary NGAL represents a much more sensitive, specific measure of kidney damage than serum NGAL.

In our use of urinary NGAL we corrected values to those of urinary creatinine in the same samples. Urinary creatinine is a widely accepted method of urinary biomarker normalization. It enables the correction of changes in the urinary biomarker concentration caused by differences in fluid administration and any diuretic use, and it is routinely used in clinical medicine. Examples include the urine microalbumin-to-creatinine ratio to detect diabetic nephropathy and the urine protein-to-creatinine ratio to quantitate proteinuria.

In a recent study NGAL was measured along with other biomarkers in renal pelvic urine at pyeloplasty.[10] This urinary NGAL level was increased compared to that in voided urine samples of control patients. Also, these renal urinary NGAL levels promptly returned to normal after repair, as determined in urine collecting from an externalized nephroureteral catheter. The investigators concluded that uNGAL could be used to identify obstruction. However, they failed to correlate these levels with any objective data indicating the relative level of function in the operated renal units. Although the reliability of diuretic renography data, such as drainage $T_{1/2}$ and differential renal function, for making surgical decisions is debatable, these data are the closest that we currently have to a standard for surgical decision making. Therefore, our study aim was to correlate information from uNGAL levels collected at surgery with preoperative data that led to the decision to perform surgery.

To validate the fact that there was a reasonable rationale for investigating uNGAL in this population, we recruited a control cohort of unaffected children. We compared bladder uNGAL levels in our study patients treated with dismembered pyeloplasty to those in unaffected controls and observed a statistically significantly higher level in patients. As suspected and as noted by Madsen et al,[10] we also observed that higher renal pelvic uNGAL correlated with lower ipsilateral differential renal function in study patients. Potentially most clinically important, bladder uNGAL levels correlated with lower differential function. This finding may indicate the usefulness of bladder uNGAL as a noninvasive means by which we could assess which patients would be at risk for decreasing renal function and be better served by intervention. The next step is to recruit enough study and control patients to reasonably identify normal uNGAL levels. Currently, the lack of such a parameter limits the clinical usefulness of NGAL in the UPJO setting.

Part of our hypothesis that failed to meet expectations in this population was the notion that more complete UPJO would lead to a uNGAL concentration in the renal pelvis. This increased ratio would indicate more complete obstruction and correlate with lower ipsilateral differential function. Interestingly, while we observed a statistically significant correlation of higher bladder and renal pelvic uNGAL levels with decreased ipsilateral function, we identified no similar relationship with the ratio. This may indicate that since bladder uNGAL is also increased when there is renal injury, the ratio may not reflect the severity of obstruction.

Clearly, we would also have wished that the correlation coefficient between uNGAL levels and differential renal function would be higher, revealing a stronger relationship. However, the correlation is statistically significant. In terms of clinical significance, we included the correlation coefficient of $T_{1/2}$ to differential renal function in our population. This was done as a comparison since $T_{1/2}$ is often clinically used in this setting, and to show that not all clinically useful parameters have a high correlation coefficient. In addition, due to the wide range of uNGAL levels in our population, we used nonparametric statistical analysis to decrease the impact of outliers. However, this may have also decreased the absolute value of the correlation coefficient.

An obvious limitation of this study is the small sample size and its single institutional nature. Further, it would be helpful to correlate uNGAL levels with clinical end points at followup. While a comparison to controls is helpful to show that bladder uNGAL is higher in patients with unilateral UPJO, it would have been more useful if control specimens had been more closely matched for age. Not having age matched controls is a study limitation. However, given that recruitment of such a young, unaffected, age matched population was difficult and the current analysis demonstrated no correlation between age and uNGAL in our population, we proceeded with data evaluation and presentation. In recognition of this limitation, we have continued recruitment of such age matched controls at our institution. Another study limitation is that surgical indications were not standardized across participating surgeons. However, this is only indicative of our prospective, observational study design.

As with any urinary biomarker for renal disease, a noninvasive method of collection would increase its clinical usefulness. In other words, a voided or bag specimen must be able to show whether the kidney in question is at risk for deterioration with conservative management. If the marker is only increased in the renal pelvis so that the specimen must be obtained via percutaneous access or surgery, the decision to proceed with intervention has already been made. While our study does not prove a role for bladder uNGAL as a noninvasive biomarker of renal damage, it provides a foundation for further study of increased bladder uNGAL in the specific setting of UPJO and potentially in other diseases affecting renal function, such as vesicoureteral reflux.

CONCLUSIONS

Bladder uNGAL is increased in children with UPJO, and renal pelvic and bladder uNGAL levels correlate with the relative function of the affected kidney in the setting of unilateral UPJO. In our prospective study of uNGAL in patients with UPJO treated with dismembered pyeloplasty we found a statistically significant correlation between higher renal pelvic and bladder uNGAL, and decreased ipsilateral differential renal function. While these initial data require further validation, we believe that they reveal the potential of uNGAL as a biomarker of renal damage in children with UPJO.

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Abbreviations and Acronyms

NGAL	neutrophil gelatinase-associated lipocalin
$T_{1/2}$	half-time
uNGAL	normalized urinary NGAL
UPJO	ureteropelvic junction obstruction

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Table 1

Characteristics of 61 study patients and 22 controls

		Pts	Controls
Median yrs age (range/95% CI)	1.6	(0.12-18.7/3.2-5.8)	8.0 (2.1-17.3/6.6-10.6)
No. male/female	36/25		11/11
No. rt/lt side	31/30		–
Median % differential function (range/95% CI)	44.5	(12-57/39.0-44.5)	–
Median mins $T_{1/2}$ (range/95% CI)	31.2	(2.8-1,260/27.5-156.8)	–
Median ng/mg NGAL (range/95% CI):*			
Normalized† bladder	18.6	(1.4-1,650.8/33.5-169.2)	8.3(0.77-39.8/6.9-17.3)
Uncorrected bladder	5.8	(0.7-478.2/8.5-50.3)	6.1 (1.2-72.5/5.8-21.0)
Normalized† renal pelvis	26.2	(1.2-18,034.5/−39.8-1,189.7)	–
Uncorrected renal pelvis	8.3	(0.5-861.7/15.8-85.8)	–
Median renal pelvis/bladder NGAL (range/95% CI):			
Normalized†	1.5	(0.2-51.7/2.5-7.7)	–
Uncorrected	1.4	(0.1-62.5/1.3-5.4)	–

*Normalized and uncorrected renal pelvic NGAL was statistically significantly greater than normalized and uncorrected bladder NGAL (p = 0.004 and 0.02, respectively)

†Correction to creatinine in same specimen.

Table 2

Correlation of NGAL and differential function

Differential Function	Spearman ρ	p Value
Normalized* NGAL:		
Bladder	−0.359	0.004
Renal pelvis	−0.383	0.002
Renal pelvis/bladder	−0.166	0.20
$T_{1/2}$	−0.363	0.007

* Correction to creatinine in same specimen.