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Impact of successful pediatric ureteropelvic junction obstruction surgery on urinary HIP/PAP and BD-1 levels

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Abstract

Introduction: In the pediatric patient whose ureteropelvic junction obstruction (UPJO) is not always symptomatic, imaging is the most common means of detecting surgical success. There is interest, however, in other means of post-operative monitoring. A panel of antimicrobial peptides (AMPs) has been previously found to be elevated in UPJO, but the impact of surgical correction on these AMPs is unknown.

Objective: To determine if elevated levels of candidate urinary AMP biomarkers of urinary tract obstruction decrease following UPJO repair.

Study design: Pediatric patients undergoing surgical correction of an UPJO were recruited for participation. Bladder urine from uninfected consenting/assenting patients was collected immediately prior to surgery and then at least 6 months afterward. Based on prior studies demonstrating significant elevation of beta defensin 1 (BD-1), hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein (HIP/PAP), cathelicidin (LL-37), and neutrophil gelatinase-associated lipocalin (NGAL) in patients with UPJO versus control patients, we performed enzyme-linked immunosorbent assays on these four AMPs to compare their expression before and after surgical intervention. If found to significantly decrease, AMP levels were

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compared to healthy controls. AMP levels were normalized to urine creatinine. Results were analyzed with paired t test or Wilcoxon test using Graphpad software. Correlation was calculated using Pearson or Spearman correlation. A p-value of <0.05 was considered significant.

Results: 13 UPJO patients were included in this study; 9 were male (69%). Age at surgery was a median of 4.3 years (average 6.1, range 0.4–18.4 years). Follow-up urine samples were collected a median of 27.4 months after surgery (average 27.4; range 7.8–45.3 months). All 13 patients had clinical improvement and/or signs of improved hydronephrosis on post-operative imaging. HIP/PAP and BD-1 significantly decreased in post-surgical samples compared to pre-surgical samples ($p=0.02$ and 0.01 , respectively); NGAL and LL-37 did not significantly change. Overall, HIP/PAP decreased in 12 patients (92%) and BD-1 decreased in 11 patients (85%). BD-1 levels after successful repair were not different from healthy controls ($p=0.06$).

Discussion: Urinary biomarkers of obstruction should detect significant obstructive pathology as well as reflect its resolution. This would enable their use in post-operative monitoring and augment current methods of determining successful surgical outcome through imaging.

Conclusions: The AMPs HIP/PAP and BD-1 are significantly elevated in UPJO but then significantly decrease after pyeloplasty, with BD-1 returning to healthy control levels. As a result, these AMPs could serve as markers of successful surgical intervention.

Graphical Abstract

Urinary markers before (Pre) and after (Post) successful surgical correction. HIP/PAP and BD-1 expression significantly decrease upon paired analysis after successful surgical correction for ureteropelvic junction obstruction (* $p=0.0215$; ** $p=0.0052$).

Keywords

biomarkers; hydronephrosis; obstructive uropathy; ureteral obstruction

Introduction

Congenital obstructive uropathy is one of the most common causes of chronic kidney disease and need for renal transplantation in the pediatric population [1]. Timely diagnosis of urinary obstruction enables appropriate interventions with hopeful preservation of renal function and/or reversal of renal disease. Current diagnostics of urinary obstruction nearly universally rely on imaging results such as hydronephrosis on renal ultrasound and signs of delayed urinary drainage with renal dysfunction on nuclear scintigraphy. Yet, hydronephrosis is a nonspecific finding, detected in 1–5% of prenatal ultrasounds [2, 3], representing a transient finding in the majority of cases that does not require intervention [4]. Nuclear scintigraphy itself can be difficult to interpret as it is dependent on adequate renal function, can be altered by capacious renal systems, and is prone to operator variability [5]. While dynamic magnetic resonance technology is emerging as a diagnostic tool, it is limited in its accessibility.

In an effort to improve current diagnostic modalities, urinary biomarkers have been evaluated for their potential use in identifying clinically significant urinary tract obstruction. Serum markers such as creatinine are already used; however, this is often normal in patients with a healthy contralateral kidney, limiting its application in patients with unilateral obstruction [6, 7].

We have previously investigated the expression of urinary antimicrobial peptides (AMPs) in patients undergoing surgical correction of an ureteropelvic junction obstruction (UPJO). AMPs are small, cationic peptides normally produced as part of the innate immune system by the urothelium, renal intercalated cells, and phagocytes in response to urinary tract infection [8, 9]. They are expressed in non-infectious states such as hemorrhagic cystitis and acute kidney injury suggesting they may be broader markers of urinary tract pathology [10, 11]. We found certain AMPs are elevated in uninfected patients with unilateral UPJO requiring surgery as compared to age- and sex-matched healthy control patients [12], suggesting urinary AMP levels may reflect changes in the uroepithelial barrier that could be altered in the setting of obstruction and thus could be used as markers of significant obstruction. As a follow-up to this initial study, the current study investigates the expression of these AMPs after successful correction of obstruction in a subset of patients who are at least 6 months from surgical repair. The primary outcome of this study is to evaluate the post-operative expression of these same AMPs after successful surgical correction. The secondary outcome of this study is to compare the post-operative expression of any decreased AMPs to that of control patients without a history of obstruction to see if these values return to normal. Our hypothesis is that elevated levels of markers in obstruction will decrease and return to normal when the obstruction is relieved.

Materials and Methods

Institutional Review Board (IRB14–00087) approval was obtained for this study.

Urine samples from pediatric patients before (“Pre”) and after (“Post”) surgery for UPJO

Previously collected aliquots of bladder urine samples from consenting/assenting pediatric patients (18 years old) at time of pyeloplasty for presumed UPJO as previously reported as the “Obstructed Group” were used in this study [12]. These samples represent the “Pre” group. Follow-up bladder urine samples were collected in the same patients at least 6 months after surgery and all drainage tubes (e.g. stents, nephrostomy tubes, catheters) were removed. We chose to base our time of collection on when any tubes were removed in order to eliminate the possibility that inflammation or urothelial changes directly in response to a foreign body could artificially alter our AMP expression. These samples represent the “Post” group. These samples were collected by either clean catch or bagged urine. Patients were only included if follow-up urine samples were obtained >6 months from surgery and removal of all urinary drains. Patients were excluded from analysis if they demonstrated signs of UTI on urinalysis (such as anything more than trace leukocytes or positive nitrites) and/or symptomatology at time of collection.

The electronic medical record was reviewed to identify any concern for persistent symptoms after surgery. All imaging was reviewed including most recent post-operative renal ultrasound and nuclear scintigraphy, if present. A single reviewer (C.B.C.) read and graded renal ultrasounds for hydronephrosis by the Society for Fetal Urology (SFU) system. Patients were included regardless of concern for re-obstruction based on imaging or symptoms.

Control urine samples from healthy pediatric patients

A selection of urine aliquots from the previously described “Control Group” [12] were used for comparison to the “Post” group. The “Control” samples chosen were age- and sex-matched to the patients on whom we had “Post” samples. We matched patients one-to-one as close to the exact age of the Post patient but did allow up to 1-year difference in age. These samples were bladder urine collected via free void or bagged sample.

Sample processing

Urinalysis of samples was performed by dipstick (Siemens Healthcare Diagnostics, Inc., Tarrytown NY) immediately after collection, after which a protease inhibitor (Assay Assure, Sierra Molecular, Incline Village NV) was added. Samples were then processed, aliquoted, and stored at –80°C until analysis as previously described [12]. Samples were restricted to a single freeze/thaw cycle before analysis.

Measurement of urinary AMPs

Based on our prior study finding that the AMPs beta defensin 1 (BD-1), hepatocarcinomatous-intestine-pancreas/pancreatitis-associated protein (HIP/PAP), cathelicidin (LL-37), and neutrophil gelatinase-associated lipocalin (NGAL) were significantly elevated in patients with UPJO compared to controls [12], we performed

enzyme-linked immunosorbent assays (ELISA) on these four AMPs to compare their expression before (Pre) and after (Post) surgical intervention.

BD-1 was run at 1:1000 dilution (PeproTech, Rocky Hill, NJ) while HIP/PAP (Fisher Scientific, Pittsburgh, PA), LL-37 (Hycult Biotech, Plymouth Meeting, PA), and NGAL (Hycult Biotech, Plymouth Meeting, PA) were all run undiluted. ELISAs were performed in duplicate on cell-free supernatants. Due to observed variability between ELISA plate readings, corresponding comparison groups were run on the same ELISA plate. AMP levels were normalized to urine creatinine (UCr) as previously described [12] and expressed as a ratio to UCr (ng/mg).

Statistical analysis

D'Agostino-Pearson omnibus normality test determined if samples were parametric. Paired sample analysis was performed by paired t test or Wilcoxon test depending if they were parametric or nonparametric, respectively. Pearson or Spearman correlation was used to evaluate relationships between variables. A p-value of <0.05 was considered significant. GraphPad Prism7.0 (GraphPad Software Inc., La Jolla, CA) was used for these analyses. Specificity was calculated using https://www.medcalc.org/calc/diagnostic_test.php.

Results

Of the 30 patients included in the original study, we were able to collect follow-up urine samples >6 months after surgery on 13 of these patients. Reasons for inability to collect follow-up samples on the other 17 patients originally included in our first study include lack of follow-up, follow-up at an off-site location that precluded being able to properly collect and process urine for analysis, and inability of the patient or unwillingness to provide a urine sample at follow-up visit. Patient demographics of those included are listed in Table 1. As stated in our original study, patients had undergone prior pyeloplasty for UPJO ultimately at the discretion of the surgical provider but for such indications as worsening hydronephrosis on renal ultrasound, decline in ipsilateral relative renal function on renal scintigraphy, and for symptoms such as pain. One of the patients included in this study had a functionally solitary kidney while all others had a normal contralateral kidney. All 13 patients had clinical improvement from before surgery and/or signs of improved hydronephrosis on post-operative imaging. One patient with symptom improvement did not have imaging available for review. None of the patients had preoperative urinary tract infections; only one patient had a post-operative urinary tract infection, 11 months before their Post sample was collected.

HIP/PAP and BD-1 significantly decreased in post-surgical samples (Post) compared to pre-surgical samples (Pre) ($p=0.0215$ and 0.0052 , respectively). NGAL and LL-37 did not significantly change ($p>0.9999$ and $p=0.5417$, respectively). We did check to see if there was a correlation between NGAL and Post SFU grade as we had found a prior correlation between Pre SFU grade and NGAL [12]. There was no significant correlation ($p=0.653$, Spearman correlation). Interestingly, in our one patient with a functionally solitary kidney, all levels of markers decreased post-operatively.

In evaluating values of each individual Pre and Post surgery, there were only two individuals with a rise in HIP/PAP and/or BD-1 after surgery (Figure 1). There was no obvious unifying factor to explain the observed rise in values: one was a 7-year-old male with an 18% functioning obstructed kidney while the other was an 18-year-old female with a 43% functioning obstructed kidney. Neither patient had a history of UTIs or a post-surgical renal scan to evaluate function. Both had improved SFU grade after surgery on post-operative renal ultrasound. Using our findings that BD-1 increased in 2 patients and HIP/PAP increased in only 1 after surgery, we calculated the specificity of BD-1 at 85% and that of HIP/PAP at 92%. Given none of our patients had evidence of failed pyeloplasty (true positives for disease), we were unable to calculate a sensitivity for our markers or perform receiver operating characteristic curves.

Since HIP/PAP and BD-1 were the only two AMPs to significantly decrease in the Post samples, we chose to compare only these two AMPs to Control urine samples. Given we had previously found that HIP/PAP expression correlated with age [12], we age- ($p=0.9591$) and sex- ($p=0.5896$) matched eight of the Post urine samples to eight of our previously collected Control urine samples. BD-1 Post urine samples normalized to our cohort of healthy control urine samples while HIP/PAP did not (Figure 2). There were only 2 individuals with post-operative nuclear scintigraphy demonstrating renal function, thus limiting our ability to check a correlation between post-operative biomarker values and their corresponding renal function. Given the variability in time from surgery, we did check for a correlation in expression of our AMPs with time. We did not find a significant correlation between Post urine levels of HIP/PAP, BD-1, NGAL, or LL-37 and time from surgery ($p=0.591$ (Spearman's correlation); $p=0.449$ (Pearson's correlation); $p=0.176$ (Spearman's correlation); $p=1.000$ (Spearman's correlation), respectively).

Discussion

Current tools for evaluation of successful surgical intervention of UPJO include imaging and clinical presentation. Pediatric patients are not always symptomatic from their UPJO to enable determination if resolution signifies improvement of obstruction and there are limitations associated with post-operative imaging [13, 14]. As such, an alternative and objective means of tracking an obstructive process in the urinary tract is attractive.

There has been great interest in identifying urinary biomarkers of pediatric urinary tract obstruction to supplement if not replace current diagnostic methods as minimally invasive alternatives to current radiologic tests [15]. We previously identified that the AMPs HIP/PAP, BD-1, NGAL, and LL-37 were significantly elevated in children with UPJO compared to age- and sex-matched controls [12], suggesting that urinary AMPs may represent novel markers of urinary tract obstruction. Given a good biomarker should also reflect its resolution [16], we performed a subsequent study to determine whether successful surgical repair led to a decrease and even normalization of HIP/PAP, BD-1, NGAL, and LL-37 urinary levels.

Of our initial four biomarkers, two suggested clinical viability. We found that HIP/PAP and BD-1 did significantly decrease after successful surgical correction with relatively high

specificity for surgical success. Unfortunately, due to the lack of surgical failures, we were unable to evaluate the sensitivity of these markers, and thus their predictive value. HIP/PAP is made specifically by the urothelium [11, 17] while BD-1 is primarily made by the renal distal tubules and collecting duct [18, 19]. Given urinary tract obstruction is known to result in epithelial injury [20, 21], it seems appropriate that peptides made by these cells may be released in the setting of obstructive uropathy. Just as their elevated expression could be a marker of obstruction, their declining expression post-operatively could be a marker of surgical success – suggesting HIP/PAP and BD-1 are candidate biomarkers for UPJO diagnosis and repair. The ability of these markers to decrease does speak towards the capability of urinary tract epithelial cells to remodel and even repair once obstruction is corrected, particularly in the case of BD-1 levels that in fact normalize to that of healthy controls.

Conversely, NGAL and LL-37, which we had previously identified as elevated in UPJO patients [12] did not significantly decrease post-operatively. NGAL serves as a marker of renal injury as a result of both increased distal nephron gene expression and altered proximal tubule reabsorption [22–24]. LL-37 is expressed by urinary tract epithelial cells [25]. The persistent elevation of these markers suggests there may be an element of irreversible or ongoing tubular damage despite apparent surgical success based on ultrasound imaging and clinical history. It would be interesting to evaluate these patients with post-operative renal scintigraphy to identify any decline in post-operative renal function. Unfortunately, only two patients in the study underwent post-operative renal scintigraphy, limiting our ability to evaluate for ongoing or irreversible renal dysfunction. This also limited our ability to correlate expression of our markers with recoverable renal function. Of note, NGAL did not correlate with preoperative relative renal function in our prior study but was associated with SFU grade ($p=0.001$) [12]. Interestingly, it did not correlate with Post SFU grade in this study.

NGAL has been demonstrated to decrease significantly as early as one month after successful UPJO repair [26, 27]. Its ability to normalize however is somewhat mixed with two studies finding that urine NGAL levels normalize to levels detected in controls by 6 months after surgery and remains persistently low 1 and 3 years after surgery [24, 26]. Another, however, found that while NGAL significantly decreases 3 months after surgery ($p<0.05$), it is still higher than in healthy children ($p>0.05$) [28]. Interestingly, Xie et al found that urinary NGAL levels measured 72 hours after surgical correction of obstructing nephrolithiasis discriminated patients with deteriorated versus stable renal function one year later [29]. Their study is novel in that it found NGAL could predict those with irreversible renal dysfunction post-operatively; prior work had simply demonstrated NGAL levels inversely correlate with the relative function of the affected kidney at the time of collection [22, 28, 30]. Future studies evaluating patients at the time of pyeloplasty for UPJO may evaluate if urinary NGAL levels could predict functional recovery in patients, particularly if an indication for intervention was diminished renal function on nuclear scintigraphy. Unfortunately, we do not have the functional data nor true positives of surgical failure to do this with the current cohort. In addition, it is unclear why we did not see a significant decrease in NGAL levels postoperatively. It is difficult to know if abnormal post-operative

function could be playing a role in our results given we do not have this data. In addition, it is possible our sample size was simply too small to detect a significant change.

We acknowledge the following limitations of our study: 1) Our sample size is small, but we found it important to follow patients past what one would expect to be the initial healing process and thus only wanted to include original participants in whom we were able to obtain repeat urine samples >6 months after surgery. In addition, our follow-up time of urine collection was highly variable. Ideally this should have been better controlled to eliminate the possibility of AMP expression changing over time. Future studies would follow expression of BD-1 and HIP/PAP over serially collected urine samples. 2) We intended to include patients regardless of their clinical sequelae, preferring to include some patients who did not have successful surgery. All patients included happened to do well clinically and radiographically. In the future, we would try to include those with “failed” pyeloplasty or with concern for re-obstruction for comparison of AMP expression. 3) We encountered inconsistencies in running ELISAs on different plates and thus ran comparable groups on the same plate. This does draw some concern for reproducibility between plate readings for these tests, as it is not practical to expect that in the development of their use for diagnostic purposes, they can be run with matching control samples.

Conclusions

Just as elevated urinary HIP/PAP and BD-1 levels suggest clinically significant upper urinary tract obstruction, their decrease suggests successful surgical intervention. Potentially, this could have implications for simplifying the required post-operative monitoring and follow-up of patients after pyeloplasty.

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Abbreviations:

AMP	antimicrobial peptide
BD-1	beta defensin 1
ELISA	enzyme-linked immunosorbent assays
HIP/PAP	hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein
LL-37	cathelicidin
NGAL	neutrophil gelatinase-associated lipocalin
SFU	Society for Fetal Urology
UCr	urine creatinine
UPJO	ureteropelvic junction obstruction

UTI urinary tract infection

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Figure 1:
Before-after graphs of individual patients before (Pre) and after (Post) surgery for each AMP.

Figure 2: Comparison of urinary BD-1 and HIP/PAP between patients who have been successfully unobstructed (Post) and age- and sex-matched healthy controls (Control). BD-1 expression normalizes after surgery without a significant difference in Post urine samples compared to Control samples ($p=0.0582$). Post urinary HIP/PAP was still significantly higher than Control samples ($\#p=0.0281$).

Table 1:

Demographics of obstructed patients with follow-up urine samples ("Post patients")

Demographic	Post patients (n=13)
Median age at surgery in years (mean; range)	4.3 (6.1; 0.4–18.4)
Median age at follow-up urine years (mean; range)	7.4 (8.3; 2.2–19.2)
Male (%)	9 (69)
Median time from surgery in months (mean; range)	27.4 (27.4; 7.8–45.3)
Clinical/radiologic improvement postoperatively (%)	13 (100)
Imaging improvement	12
Imaging resolution	2
Symptom improvement	1
Median preoperative SFU grade hydronephrosis (mean; range)	4 (3.8; 3–4)
Median postoperative SFU grade hydronephrosis (mean; range)	2 (1.6; 0–2)