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Urinary NGAL Deficiency in Recurrent Urinary Tract Infections

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Abstract

Introduction—Children with recurrent urinary tract infections (rUTI) often do not have an identifiable cause of their infections. Neutrophil gelatinase-associated lipocalin (NGAL) is known to be upregulated within the uroepithelium and kidney of patients with UTI and exhibits a localized bacteriostatic effect through iron chelation. We hypothesize that some patients with rUTI without an identifiable cause of their recurrent infections are locally deficient for NGAL production.

Materials and Methods—Patients seen in the urology clinic for rUTI who were under 21 years of age were enrolled. Patients were excluded if they had UTI at the time of enrollment, evidence of renal disease, decreased renal function, known anatomic abnormality of the genitourinary tract or other reason that predisposes to UTI, such as requirement for intermittent catheterization, neurogenic bladder, or unrepaired posterior urethral valves. Control patients were healthy children enrolled from the emergency department, and were included if they no history of UTI or renal dysfunction, a normal urinalysis at the time of enrollment, and were not presenting with diagnosis associated with increased NGAL levels, such as acute kidney injury or infection. NGAL was measured by immunoblot.

Results—5 cases and controls were enrolled. Median urinary NGAL levels were significantly decreased in rUTI patients compared to controls (15 (14,29) ng/ml vs 30 (27,61) ng/ml, p=0.002). Although comparatively diminished, measurable NGAL levels were present in all patients with rUTI.

Discussion—Here, we explored the hypothesis that a lack of NGAL production may be a factor in the pathogenesis of rUTI. While there are several studies investigating the role of NGAL both in UTI and in iron trafficking within the genitourinary environment, there are no previous works that explore the concept of NGAL deficiency. There are several limitations to this study, mostly related to the exploratory nature of this investigation. The limitations of this study include the lack of matching between cases and controls, and the small number of patients in the cohorts.

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Conclusions—Urinary NGAL is significantly decreased in patients with rUTI compared to patients without rUTI. These data suggest that some patients with rUTI may be predisposed to UTI because of a relative local deficiency in urinary NGAL production.

Introduction

Urinary tract infections (UTI) are common within the pediatric population. Recurrent UTIs (rUTI) occur in approximately 12% of patients following an initial UTI.[1] The pathophysiology of rUTI is multifactorial, but is not fully understood. Deficiencies in the innate immune system are likely involved.

Neutrophil Gelatinase-Associated Lipocalin (NGAL), a protein expressed in the uroepithelium,[2] functions within the innate immune system by exerting a bacteriostatic effect on gram-negative bacteria through iron chelation.[3] Urinary NGAL (uNGAL) has been shown to be variably upregulated in UTI.[4] Given the role of NGAL in bacteriostasis and its upregulation in UTI, we sought to determine if a subset of patients with rUTI had a deficiency in uNGAL.

Methods

This study was approved by the Boston Children's Hospital Institutional Review Board, and informed consent was obtained. Cases were patients between 6 months and 21 years of age with a history of rUTI who were prospectively identified from the Department of Urology at Boston Children's Hospital from February 2013 through August 2013. RUTI was defined by two or more culture proven UTIs with at least 100,000 cfu/mL of a single uropathogen. Cases were excluded if there was suspicion of an active UTI as evidenced by either clinical symptoms, the presence of leukocyte esterase or nitrites in the urinalysis, or any bacterial growth (>10,000 cfu/mL) in urine cultures, or if they had renal dysfunction, or had a UTI within 30 days prior to enrollment. 18 patients were initially enrolled as cases, 3 that were excluded due to positive urine cultures at the time of enrollment.

Control patients were a convenience sample enrolled from the Emergency Department at Boston Children's Hospital. Patients who provided urine samples for routine clinical purposes were approached for inclusion in this study as controls. None of the control patients required fluid resuscitation or hospital admission, or had any clinical parameters to suggest dehydration, such as tachycardia or decreased urine output. Controls were excluded if they had a history of a UTI, evidence of a UTI at the time of enrollment (defined above), known or suspected renal dysfunction, or had any urologic history. In an effort to enroll otherwise healthy children as controls, patients followed by any subspecialists were not included as controls. This was determined through a combination of parental report and chart review. 15 patients were enrolled as controls, of which 53% had a urine sample sent for a pregnancy test prior to radiologic imaging for an orthopedic injury, 33% presented with constipation with relief of symptoms following enema, and 13% presented with headache.

Samples were centrifuged at 12,000 RPM for 5 minutes, aliquoted and stored at -80 degrees Celsius within 12 hours of collection. UNGAL measurements were performed in duplicate by western blot with 10μL samples. Non-reducing 4–20% gradient polyacyrlamide gels

(Bio-Rad, Hercules CA) were used for electrophoresis. UNGAL was detected with monoclonal antibodies to NGAL (1:1000, Antibody Shop, BioPorta Diagnostics, Gentofte, Denmark), and with rabbit polyclonal secondary antibody (Jackson ImmunoResearch, West Grove PA). Standards of human recombinant NGAL were used to standardize quantification. [3]

Patient data was collected from the electronic medical record. Data analysis was performed using SPSS 16.0 (SPSS, Chicago, Illinois). We compared normally distributed continuous variables using a 2-tailed Student's T test, and categorical data by using chi-square tests, rejecting the null hypothesis at a significance of 0.05. Non-normally distributed variables, i.e. uNGAL, were compared using the Mann-Whitney-U test.

Results

There were 15 patients in each group. There were more females in the rUTI group compared to the control group (100% (15/15) vs. 87% (13/15), $p<0.01$). Patients in the control group were older than those with rUTI (13.6±5.0 vs. 8.0±3.9 years, $p=0.02$) (Table 1). All patients in the rUTI group all underwent some type of radiologic imaging (Table 1). 87% (13/15) of the renal ultrasounds were normal, with the remaining identifying minimal and non-clinically relevant pelviectasis. One patient had grade II vesicoureteral reflux, and both DMSA studies identified cortical defects. Median uNGAL levels were significantly lower in the rUTI patient cohort (15 (14,29) ng/ml vs 30 (27,61) ng/ml, $p=0.002$).

Discussion

In this study we demonstrated that patients with rUTI have significantly lower baseline uNGAL levels compared to healthy controls. The pathogenesis of rUTI is complex and heterogeneous. Various authors have investigated the genetic basis of rUTI, and have identified several genes within the innate immune system that play a role in recurrent infection.[5],[6] Reduced toll-like receptor 4 (TLR4) expression has been suggested to promote an asymptomatic bacteriurial state.[7] TLR-4 is activated by lipopolysaccharides, which is found on the outer membrane of gram-negative bacteria, and induces NGAL expression in the alpha-intercalated cell.[8] This activation is essential in the innate immune response by activation of proinflammatory pathways and induction of cytokine expression.

NGAL exerts a bacteriostatic effect in the urinary tract by chelating iron from gram-negative bacteria. NGAL deficiency would potentially promote bacterial growth, increasing the bacterial burden within the urologic system and increasing the risk of infection. Here, we demonstrated that patients with rUTI have significantly lower uNGAL levels compared to healthy controls. To date, no studies investigated the hypothesis that baseline uNGAL deficiency may predispose to rUTI. Previous studies have shown that NGAL is increased in acute infections of the urinary tract and that uNGAL levels demonstrated a dose-response relationship to bacterial colony counts.[4][9] These studies were cross-sectional and measured NGAL levels during active infection. In contrast to this study, our cohorts were measured during a period of quiescence, the lower baseline level may be indicative of the increased risk for recurrence.

The decision to not standardize uNGAL with urine creatinine is based on the biology of the NGAL. Although standardization with urine creatinine is a common practice within the NGAL literature, we believe this is an imperfect standardization. NGAL is primarily produced within the collecting ducts and is essentially independent from glomerular filtration, whereas urine creatinine comes from the filtrate at the level of the glomerulus and represents a distinct biology than that of urine NGAL. Despite the relatively small size of NGAL, prior work has demonstrated that serum NGAL is not filtered by the glomerulus. Indeed, even when large amounts of NGAL are injected into healthy mice, almost none is subsequently found in the urine. This is likely due to the fact that NGAL is absorbed by megalin, a protein in the serum. When large amounts of NGAL are injected into a megalin knock-out mouse, significantly increased NGAL levels are found within the urine.[10]

33% of the patients included as controls within our cohort who were diagnosed with constipation. While the association between the dysfunctional elimination syndrome (DES) and UTIs in children has been well documented, DES is common, and has not been shown to be a causative factor in UTIs.[11] Indeed, the rate of DES is the same between young children with and without history of UTI.[12] Further, prevalence of UTI was not higher in children with constipation compared to children without constipation.[13] Finally, within a cohort of children with constipation, lack of development of a UTI would suggest the presence of a normally functioning innate immune system, which is the goal of the control group within this study.

Although our findings of decreased uNGAL in patients with rUTI is intriguing, this pilot study has limitations including: small cohorts, the composition of the cohorts, a single-center design, and age difference between the cases and controls. As in all comparative studies, obtaining optimal age and sex matched control cohort is challenging. A cohort of 368 healthy children showed that NGAL levels are significantly elevated in those ages 10–<15 years, which include 13.6 years, the mean age of our control group, compared to children ages 5–<10 years, which include 8.0 years, the mean age of our rUTI group. However, while statistically significant, this difference is small (7.6ng/ml vs 4.5 ng/ml), and is likely not a significant contribution. Conversely, the same study shows that females, who make up 100% of our rUTI group and 86.7% of our control group, have a significantly higher level of NGAL compared to males, 13.5ng/ml versus 3.5ng/ml.[14] Therefore, while there are differences in NGAL levels based on age and gender, they are minimal and not likely to significantly affect our results.

Overall, we demonstrated an association between rUTI and a decreased level of uNGAL during an asymptomatic period. Although mechanistically appealing, no causality can be inferred from this data. It is possible that the decreased NGAL levels are secondary to renal scarring as a result of rUTI, and therefore the decreased NGAL levels are a result of rUTI. However, NGAL levels are increased in patients with renal scarring compared to those without, suggesting that renal scarring may not be a cause of decreased NGAL levels.[15] Confirmation of these results in a larger prospective cohort, with well-matched controls, will help to further elucidate the relationship between NGAL levels and rUTI.

Conclusion

Baseline uNGAL levels during a clinically asymptomatic period were significantly lower in a pediatric patients with rUTI as compared to healthy controls. Lower uNGAL may either be the cause or the effect of the rUTI.

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Abbreviations

UTI	urinary tract infection
rUTI	recurrent urinary tract infection
NGAL	neutrophil gelatinase-associated lipocalin
uNGAL	urinary neutrophil gelatinase-associated lipocalin
TRL4	toll-like receptor 4

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Table 1

Patient Demographics, Imagining, and NGAL Levels

	rUTI (n=15)	Controls (n=15)	p-value
Age (years)	8.0 (3.9)	13.6 (5.0)	0.02
Sex (% female)	100	86.7	<0.01
Race (% white)	50	33.3	0.44
Urine pH	6.8 (0.9)	6.4 (1.0)	0.31
Family History of UTI	20%	–	ND
Renal Ultrasound	100%	–	ND
VCUG	67%	–	ND
DMSA Scan	13%	–	ND
Median uNGAL ng/mL (IQR)	15 (14,29)	30 (27,61)	0.002

ND = not done, VCUG = voiding cystourethrogram

Table 1: Demographic information, imaging studies, and uNGAL levels regarding patients with rUTI and controls. Data presented as mean (standard deviation), percentages, or median (interquartile range) as stated.