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Periurethral colonization and urinary leukocytes as markers for bacteriuria in children with neurogenic bladder

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Summary. Bacteriuria and associated renal damage is common in children with a neurogenic bladder, but the pathogenesis of urinary tract infection (UTI) is undefined. We examined the association between periurethral bacterial colonization and the presence of urinary leukocytes in 76 catheter urine specimens from children with neurogenic bladders. Although all the children were asymptomatic, 38/76 (50%) of the urine cultures were positive. Periurethral colonization was significantly more common with positive than with negative urine cultures, suggesting a pathogenetic role for periurethral bacteria in infection of the neurogenic bladder. Urinary leukocytes were present in 24/38 (63%) with positive cultures, as against none (0/38) of those with negative urine cultures, and their presence represents a host response to bladder bacteriuria.

Key words: Bacteriuria – Urinary tract infection – Neurogenic bladder

Although bacteriuria and its associated risk of renal damage is a major problem in children with neurogenic bladder, the pathogenesis of urinary tract infection (UTI) is undefined [3–5]. It has been well established that school girls with „asymptomatic bacteriuria" and normal bladder function are at low risk for UTI and the development of renal damage [8]. However, the significance of bacteriuria is difficult to assess in children with neurogenic bladder because the child's neurologic impairment may mask the usual symptoms of UTI. As a result, these children are often treated with prolonged courses of antibiotics, which has been associated with selection of resistant organisms and toxic side effects.

The sequence of events leading to infection of the normal bladder is thought to begin when bacteria on the periurethral mucosa ascend into the bladder and subsequently infect the urine or bladder mucosa. Periurethral colonization with gram-negative rods is more common in girls and women with normal bladder function and recurrent UTI than in controls [2, 11]. However, the role of periurethral colonization in infection of the neurogenic bladder has not been defined. As a first step in defining the pathogenesis of UTI, we documented the association of periurethral colonization with bacteriuria in children with neurogenic bladder. In addition, we examined whether the presence of urinary leukocytes represented a host response to infection in a population with neurologic impairment.

Methods

Fifty children and young people, 17 male and 33 female (3 months to 21 years of age), with neurogenic bladder were evaluated during "well visits" to the Myelomeningocele Clinic, Children's Rehabilitation Center, University of Virginia Medical Center from July 1989 to June 1990. Neurogenic bladder was due to myelomeningocele in 48 and in 2 to traumatic spinal cord injury. All of the children lived at home, had clean intermittent catheterization or diaper as their voiding technique and had a normal urinary tract according to abdominal ultrasound. At each visit informed consent was obtained from the legal guardian for study and symptoms or signs of UTI (fever, abdominal pain, change in continence pattern, or change in color/odor of urine) were recorded (American Academy of Cerebral Palsy and Developmental Medicine Round Table Discussion of Symptoms of UTI in Neurogenic Bladder, October, 1990 Meeting, Orlando, Fla.).

A periurethral culture was obtained by swabbing the periurethral mucosa once with a single cotton-tipped swab after separating the vaginal labia in females or retracting the forseskin (if needed) in males. The swab was immediately broken off in a glass vial containing 2 ml of sterile saline and transported to the laboratory, where the liquid in the swab was expressed by rolling the cotton against the side of the tube in order to elute bacteria into the saline. Then 10 µl of the eluate was plated onto 5% sheep blood and MacConkey agar. Plates were cultured aerobically (37°C, 24 h), and organisms identified by standard clinical laboratory methods. Periurethral colonization was defined as 10^2 or more colony forming units (cfu) of a urinary tract pathogen per ml inoculated saline.

After the periurethral culture was collected, urine was obtained by the in-and-out sterile catheterization technique. Urinalysis including a dipstick reading (Ames, Elkhart, Ind.) and microscopic examination of the urine sediment were performed according to standard procedures. For microscopy, 12 ml urine was placed in a standard centrifuge tube (Urine-Tek, Ames). Samples were spun for

Table 1. Correlation of urinalysis with culture in 76 urine samples obtained by in-and-out catheterization from 50 children with neurogenic bladder

Urinalysis	Urine Culture	
	Positive $n=38$ (%)	Negative $n=38$ (%)
Bacteria		
Bacteria on microscopy and positive nitrite	21 (55)	0
Bacteria on microscopy	31 (82)	1 (3)
Leukocytes		
≥ 5 Leukocytes/high-power field and positive leukoesterase	17 (45)	0
Positive leukoesterase	24 (63)	0

Table 2. Likelihood of urinary leukocytes in 38 positive urine cultures according to clinical and laboratory features among children with neurogenic bladder

Features	Total no. of cultures	No. (%) of specimens with urinary leukocytes
Bacterial count		
$\geq 10^5$ cfu/ml	27	18 (67)
10^4 cfu/ml	11	6 (55)
Bacterial species		
E. coli	18	9 (50)
Other than *E. coli*	20	15 (75)
Urine pH		
<6.8	18	10 (56)
≥ 6.8	20	14 (70)
Periurethral colonization[a]		
Yes	31	21 (68)
No	6	2 (33)
Voiding technique		
CIC[b]	30	18 (60)
Diaper	8	6 (75)
Prophylactic antibiotics		
Yes	10	7 870)
No	28	17 (60)

[a] A periurethral sample was obtained prior to 37 culture-positive urine specimens

[b] Clean intermittent catheterization

6 min at 2300 rpm, after which the supernatant urine was drained off. The deposit was resuspended in the last drop or two of supernatant and examined under a light microscope (40×), an average high-power field count (HPF) being made of 10 separate fields. A positive microscopy was defined as the presence of any bacteria, or greater than or equal to five leukocytes/high-power field (≥ 5 WBCs/HPF) [7]. The colony count in the urine was determined by inoculation of 5% sheep blood and MacConkey agar using a 0.001 ml calibrated loop. A urine culture was defined as positive (bladder bacteriuria) if 10^4 cfu or more of a urinary tract pathogen was present per ml urine. Inflammation of the urinary tract was defined as bladder bacteriuria with the presence of leukocytes (positive leukoesterase or ≥ 5 WBCs/HPF). For the purposes of this study urinary tract pathogens included all the Enterobacteriaceae, *Enterococcus sp.*, *Staphylococcus aureus* and group B streptococci. Quantitation and speciation of organisms along with urinalysis was performed at the Pediatric Microbiology Laboratory, University of Virginia.

Statistical analysis was done using the Fisher exact test.

Results

Seventy-six urine specimens were collected from 50 children with neurogenic bladder by in-and-out catheterization. Twenty patients had all positive urine cultures (one or more cultures), 24 patients had all negative cultures (one or more), and 6 patients had a positive culture followed by a negative culture at successive visits. There was no significant correlation between urine culture results and age or sex of the child, level of spinal cord lesion, usual voiding technique or use of prophylactic antibiotics.

All the children or their parents denied symptoms or signs of UTI; however, 38/76 (50%) of the urine cultures were positive. Enterobacteriaceae was isolated from all cultures except one, which grew an Enterococcus species. A periurethral sample was obtained before urine culture in all but one instance. Of the positive urine cultures, 31 (84%) were associated with one or more species of Enterobacteriaceae or Enterococcus on the periurethra. In contrast 14/38 (37%) negative cultures were associated with one or more periurethral pathogens. Thus, periurethral colonization was significantly more frequent in the group of children with positive urine cultures than in the children with negative cultures ($P<0.05$). There was no significant difference between the sex of the child and the detection of pathogen(s) on the periurethra.

To analyze the significance of bacteriuria in these children without symptoms of UTI, urinalyses of the 38 culture-positive and 38 culture-negative urine specimens were compared (Table 1).

Bacteria

A positive nitrite test was always associated with the presence of bacteria on microscopic examination and was positive in 21/38 (55%) positive cultures and 0/38 negative cultures. The presence of bacteria on microscopy in the face of a negative nitrite test was observed in an additional 10 positive cultures and in 1 negative culture. As expected, the 1 positive culture for *Enterococcus sp.* produced a negative nitrite test [6]. Thus a positive nitrite test or the identification of bacteria on microscopy was highly predictive of a positive culture (positive predictive value of 97%, negative predictive value of 84%).

Leukocytes

The presence of ≥ 5 WBCs/HPF on microscopic examination was always associated with a positive leukoesterase

and was observed in 17/38 (45%) positive cultures. Positive leukoesterase and no leukocytes/HPF was observed in further 7 positive cultures [9]. Thus a positive leukoesterase or ≥ 5 WBCs/HPF was highly predictive of a positive urine culture (positive predictive value of 100%). Importantly, none of the 38 culture-negative specimens had evidence of leukocytes in the urine.

To determine whether bacteriuria in the presence of urinary leukocytes (positive leukoesterase or ≥ 5 WBCs/HPF) indicative of urinary tract inflammation differed from bacteriuria alone, we examined clinical and laboratory features of the 38 positive urine cultures (Table 2). There was no significant correlation between the presence or absence of urinary leukocytes and bacterial counts (10^4 cfu/ml vs $\geq 10^5$ cfu/ml), species of bacteria, pH of urine, periurethral colonization, voiding technique or use of prophylactic antibiotics in the 38 positive cultures.

Discussion

The results of this study confirm that bacteriuria is common in children with neurogenic bladder. The absence of symptoms in all our children is not clinically important, because the majority of children have sensory deficits. Our study determined that the frequency of pathogen(s) on the periurethra in males and females was significantly greater with positive than with negative urine cultures. Our results agree with findings in female subjects with normal urinary tracts and a history of UTI [2, 11], in whom one or more species of Enterobacteriaceae were detected on the periurethra. In contrast, female subjects without a history of UTI rarely had pathogen(s) detected on their periurethra. These findings suggest that infection of the neurogenic bladder results from ascension of periurethral pathogens into the bladder urine. However, detection of a bacterial species on the periurethra and in the urine does not establish that the infecting organism came from the periurethra. If it is found by genetic analysis [10, 12] that periurethral colonization predicts subsequent bacteriuria with the same organism, then strategies could be designed to alter colonization or virulence factors of the colonizing pathogen. This study also documented that two-thirds of the positive cultures and none of the negative cultures were associated with inflammation of the urinary tract. Urinary leukocytes are a host response to the presence of bacteria in the neurogenic bladder [1]. Alternatively, bacteriuria without urinary leukocytes implies mere colonization of the bladder. Future studies will examine whether the presence of urinary leukocytes predict persistent bacteriuria and subsequent deterioration of the urinary tract in children with neurogenic bladder.

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