

## ORIGINAL CONTRIBUTION

# Use of Urinary Gram Stain for Detection of Urinary Tract Infection in Childhood

Şükrü Arslan, Hüseyin Çaksen<sup>a</sup>, Levent Rastgeldi, Abdurrahman Üner, Ahmet Faik Öner, and Dursun Odabaş

*Department of Pediatrics, Yüzüncü Yil University Faculty of Medicine, Van, Turkey*

*In this study, urinary culture, urinary Gram stain, and four tests within the urinalysis, leukocyte esterase, nitrite, microscopy for bacteria, and microscopy for pyuria, were examined in 100 children with symptoms suggesting urinary tract infection. Our purpose was to determine the validity of the urinary Gram stain compared with a combination of pyuria plus Gram stain and overall urinalysis (positiveness of nitrite, leukocyte esterase, microscopy for bacteria, or microscopy for white blood cell). Of 100 children, aged two days to 15 years, 70 (70 percent) had a positive urinary culture: 40 girls (57 percent) and 30 boys (43 percent). Escherichia coli was the most common isolated agent. The sensitivity and specificity of the urinary Gram stain were 80 percent and 83 percent, and that of the combination of pyuria plus Gram stain 42 percent and 90 percent, and that of the overall urinalysis 74 percent and 3.5 percent respectively. Our findings revealed that neither method of urine screen should substitute for a urine culture in the symptomatic patients in childhood.*

## INTRODUCTION

Urinary tract infection (UTI)<sup>b</sup> is a common disease in childhood and approximately 3 to 5 percent of girls, and 1 percent of boys develop a UTI [1-3]. In this age group, prompt treatment is essential because even a brief delay can cause permanent complications [4-6]. However, prompt treatment depends on rapid diagnosis. Although several rapid screening techniques such as urinalysis (microscopic pyuria), enhanced urinalysis (white blood cell [WBC] count per cubic millimeter plus Gram stain), urine dipstick (leukocyte

esterase or nitrite), and uriscreen (catalase test) tests have been used in diagnosis of UTI, none of them have shown to be satisfactory [4, 5, 7-9].

In this study, urinary culture, urinary Gram stain, and four tests within the urinalysis, leukocyte esterase, nitrite, microscopy for bacteria, and microscopy for pyuria, were examined in children with symptoms suggesting UTI. We aimed to determine the validity of the urinary Gram stain compared with a combination of pyuria plus Gram stain and overall urinalysis (positiveness of nitrite, leukocyte

<sup>a</sup> To whom all correspondence should be addressed: Hüseyin Çaksen, M.D., K. Karabekir C. Gölbaşı 3. S., Erkam sitesi. B Blok K: 3, No: 7 Van, Turkey. Tel.: 90-432-217-61-28; Fax: 90- 432-215-04-79; E-mail: huseyincaksen@hotmail.com.

<sup>b</sup> Abbreviations: UTI, urinary tract infection; WBC, white blood cell.

Received: June 20, 2001; Returned for revision: January 24, 2002; Accepted: June 1, 2002.

esterase, microscopy for bacteria, or microscopy for WBC).

## MATERIALS AND METHODS

The study included 100 children who were admitted to the Yüzüncü Yil University, Faculty of Medicine, Department of Pediatrics, between January 1999 and June 1999 with symptoms suggesting UTI. Inclusion criteria were: for infants, fever with no apparent source, vomiting, decreased appetite, and irritability; for toddlers, abdominal pain and voiding frequency with or without fever; and for older children, dysuria, frequency, urgency, and abdominal/flank pain with or without fever. Children receiving antibiotic therapy were excluded from the study.

In all children, physical examination was performed, and four tests within the urinalysis (leukocyte esterase, nitrite, microscopy for bacteria, and microscopy for pyuria), urinary Gram stain, and urinary culture were examined. Complete blood count, peripheral blood smear and erythrocyte sedimentation rate were also analyzed. The children diagnosed as UTI were treated with appropriate antibiotic to the result of antibiogram.

In infants, the application of an adhesive, sealed, sterile collecting bag after disinfection of the skin of the genitals was used for obtaining urine. In toilet-trained children, a midstream urine sample was taken [1].

Urinalysis leukocyte esterase and nitrite studies were performed in fresh and uncentrifuged urine by using an automated urine analyzer (IRIS 500™, IRIS Company, Los Angeles, California). Microscopy for bacteria and pyuria was performed on a centrifuged urine specimen in all children. Within automated urinalysis, leukocyte esterase was considered positive if at least a "trace" was present; nitrite was read qualitatively as "positive" or "negative." Pyuria

was considered present if more than five WBCs were noted on unstained microscopy, and bacteriuria was present if at least a "slight" reading was noted per average high-powered field (40x magnification). In an effort to augment the sensitivity of urinalysis, an "overall urinalysis" test was conceived, which was considered positive if nitrite, leukocyte esterase, direct microscopy without Gram stain for bacteria, or microscopy for WBC was positive. Gram stains were positive if any organisms were present on a survey of 20 oil immersion fields.

Quantitative urine culture was performed, using a loop calibrated to deliver 0.01 ml to inoculate sheep blood agar and eosine metilen blue agar culture plates. All plates were incubated at 35°C and read at 24 and 48 hours for bacterial identification and colony count. Gram stains were performed on all specimens using of centrifuged urine. Cultures were considered positive if the culture showed greater than 100,000 colonies of a single pathogen [1].

The results were analyzed for Gram stain alone, a combination of pyuria plus Gram stain, and overall urinalysis. With a positive urine culture of more than 100,000 pure growth organisms per milliliter as the validating standard, sensitivity, specificity, and positive and negative predictive values were calculated for Gram stain alone, a combination of pyuria plus Gram stain, and overall urinalysis.

Statistical analysis was performed by using the  $\chi$ -square test (p value of less than 0.05 was considered statistically significant).

## RESULTS

A total of 100 children, aged two days to 15 years, were included in the study: 52 girls (52 percent) and 48 boys (48 percent). Of these children, 70 (70 percent) had a positive urinary culture, 40 girls (57 percent) and 30 boys (43 percent). Most of

**Table 1. Data of the children with positive cultures according to the age and gender.**

Age groups	Male n (%)	Female n (%)	Total n (%)
0 to 12 months	19 (64)	12 (30)	31 (44)
13 months to 5 years	11 (36)	17 (42.5)	28 (40)
6 to 10 years	0 (0)	9 (22.5)	9 (13)
11 to 15 years	0 (0)	2 (5)	2 (3)
Total	30 (100)	40 (100)	70 (100)

$$\chi^2 = 12.70; p < 0.05$$

these children were younger than five years old. There is a significant difference between the age groups and gender ( $p < .05$ ) (Table 1). Of the cultures, 33 (47 percent) were positive for *Escherichia coli*, 13 (18.5 percent) for *Klebsiella pneumonia*, seven (10 percent) for *Proteus mirabilis*, and six (8.5 percent) for *Staphylococci*, etc. (Table 2). Table 3 summarizes the clinical and laboratory features of the children with positive and negative urinary cultures. There is not a significant difference between the age groups and culture positiveness ( $\chi^2 3.27; p > 0.05$ ).

Table 4 summarizes sensitivity, specificity, and predictive values for Gram stain, a combination of pyuria plus Gram

stain, and overall urinalysis. While the highest (80 percent) sensitivity was determined in urinary Gram stain, the highest (90 percent) specificity was observed in the combination of pyuria plus Gram stain.

## DISCUSSION

Although a number of studies have evaluated the use of urinary Gram stain in infants and children, the results obtained from the investigations are fairly different [9-12]. A study by Lochart et al. [9] of 207 infants less than six months of age with fever found the Gram stain to have a sensitivity of 94 percent and specificity of 92 percent, and overall urinalysis test to have

**Table 2. Distribution of patients according to the bacteria isolated from urine cultures.**

Bacteria isolated from urine cultures	Male n (%)	Female n (%)	Total n (%)
<i>Escherichia coli</i>	7 (23)	27 (67.5)	33 (47)
<i>Klebsiella pneumonia</i>	6 (20)	9 (22.5)	13 (18.5)
<i>Proteus mirabilis</i>	6 (20)	0 (0)	7 (10)
<i>Staphylococci</i>	4 (13)	0 (0)	6 (8.5)
<i>Enterobacter</i>	3 (10)	2 (5)	5 (7)
<i>Streptococci</i>	1 (3.5)	1 (2.5)	2 (3)
<i>Pseudomonas aeruginosa</i>	1 (3.5)	1 (2.5)	2 (3)
<i>Serratia marcescens</i>	1 (3.5)	0 (0)	1 (1.5)
<i>Morganella morganii</i>	1 (3.5)	0 (0)	1 (1.5)
Total	30 (100)	40 (100)	70 (100)

**Table 3. The clinical and laboratory features of children with positive and negative urinary cultures.**

Parameters	Cases of positive culture n (%)	Cases of negative culture n (%)	Total n (%)
<b>Age</b>			
0 to 12 months			
Female	12 (17)	4 (13.5)	16 (30.5)
Male	19 (27)	5 (16.5)	24 (43.5)
13 months to 5 years			
Female	17 (24.5)	5 (16.5)	22 (43)
Male	11 (15.5)	7 (23.5)	18 (39)
6 to 10 years			
Female	9 (13)	3 (10)	12 (21)
Male	0 (0)	4 (13.5)	4 (13.5)
11 to 15 years			
Female	2 (3)	0 (0)	2 (3)
Male	0 (0)	2 (6.5)	2 (6.5)
<b>Urinary dipstick examination</b>			
Nitrite			
Positive	29 (41.5)	12 (40)	41 (41)
Negative	41 (58.5)	18 (60)	59 (59)
Leukocyte esterase			
Positive	46 (66)	23 (76.5)	69 (69)
Negative	24 (34)	7 (23.5)	31 (31)
<b>Microscopic examination of urine</b>			
Pyuria			
Yes	35 (50)	22 (73)	57 (57)
No	35 (50)	8 (27)	43 (43)
Bacteriuria			
Yes	38 (54)	20 (65.5)	58 (58)
No	32 (46)	10 (34.5)	42 (43)
<b>Urinary Gram stain</b>			
Bacteria present	56 (80)	5 (16.5)	61 (61)
Bacteria absent	14 (20)	25 (83.5)	39 (39)
Total	70 (100)	30 (100)	100 (100)

a sensitivity of 67 percent and specificity of 79 percent. A review of 1,019 symptomatic pediatric outpatients showed that Gram-stained smear was slightly more sensitive than dipstick (either leukocyte esterase-or nitrite-positive); 97.6 percent vs. 90.2 percent, but the positive predictive value was low for both methods [11]. Hoberman and coworkers [12] reported 96 percent sensitivity and 93 percent specificity for the enhanced urinalysis, and they also noted that the presence of either

pyuria or bacteriuria and the presence of both pyuria and bacteriuria have the highest sensitivity (95 percent) and positive predictive value (85 percent). Shaw et al. [10] noted enhanced urinalysis was most sensitive (94 percent) at detecting UTI but had more false-positive results (16 percent) than the urine dipstick (leukocyte esterase or nitrite) or Gram stain (3 percent).

Because we did not count leukocytes per cubic millimeter in urine samples, we

**Table 4. Sensitivity, specificity, and predictive values for Gram stain, enhanced urinalysis, and overall urinalysis.**

	Gram stain <sup>a</sup>			Combination of pyuria and Gram stain <sup>b</sup>			Overall urinalysis <sup>c</sup>		
	Positive culture	Negative culture	Total	Positive culture	Negative culture	Total	Positive culture	Negative culture	Total
Positive test	56	5	61	30	3	33	52	29	81
Negative test	14	25	39	40	27	67	18	1	19
Total	70	30	100	70	30	100	70	30	100

<sup>a</sup> Sensitivity, 80%; specificity, 83%; positive predictive value, 91%; negative predictive value, 64%.

<sup>b</sup> Sensitivity, 42%; specificity, 90%; positive predictive value, 90%; negative predictive value, 40%.

<sup>c</sup> Sensitivity, 74%; specificity, 3.5%; positive predictive value, 64%; negative predictive value, 5%.

could not comment on enhanced urinalysis. We only recorded the number of leukocytes and bacteria per average high-powered field, and the highest sensitivity and specificity were determined in urinary Gram stain and in the combination of pyuria plus Gram stain, respectively. The overall urinalysis appeared the lowest specificity among the tests. Our findings were partially compatible with the literature data.

Waisman et al. [7] revealed that the sensitivity and specificity of urinalysis (microscopic pyuria) were 88.6 percent and 88.4 percent, respectively, and the sensitivity and specificity of dipstick tests (leukocyte esterase and nitrite) were 97.1 percent and 82.5 percent, respectively. Matthai and Ramaswamy [13] noted that the sensitivity and specificity of bacteriuria 78 percent and 96 percent, and that of pyuria (>10 WBC/hpf) 80 percent and 82 percent, respectively. Leanos-Miranda et al. [14] stated that the combination of positive tests in leukocyte and bacteriuria increased the specificity and the positive predictive value (99 percent and 96 percent respectively). Our study did not examine the use of the microscopic urinalysis alone. The sensitivity of overall urinalysis was higher than the combination of

pyuria and Gram stain, but the specificity was the poorest.

In conclusion, our findings revealed that a combination of pyuria plus Gram showed the highest (90 percent) specificity, but the lowest (42 percent) sensitivity; urinary Gram stain demonstrated the highest (90 percent) specificity; and overall urinalysis displayed the lowest (3.5 percent) specificity. To these findings we suggest that neither method of urine screen should substitute for a urine culture in the symptomatic patients in childhood.

## REFERENCES

1. Elder, J.S. Urinary tract infections. In: Behrman, R.E., Kliegman, R.M., and Jenson, H.B., eds. *Textbook of Pediatrics*. Sixteenth edition. Philadelphia: W.B. Saunders; 2000, pp. 1621-1625.
2. Çaksen, H., Cesur, Y., Üner, A., Arslan, Ş., Şar, S., Çelebi, V., and Kuru, M. Urinary tract infection and antibiotic susceptibility in malnourished children. *Int. Urol. Nephrol.* 32:245-247, 2000.
3. Çaksen, H., Arslan, Ş., Abuhandan, M., Çelik, A., Bozkurt, H., and Odabaş, D. Asymptomatic bacteriuria in infants in eastern Turkey. *Acta Paediatr. Taiwan* 42:338-339, 2001.
4. Zelicovic, I., Adelman, R.D., and Nancarrow, P.A. Urinary tract infections in children: an update. *West. J. Med.* 157:554-561, 1992.

5. Hoberman, A. and Wald, E.R. Urinary tract infections in young febrile children. *Pediatr. Infect. Dis. J.* 16:11-17, 1997.
6. Vickers, D., Ahmad, T., and Coulthard, M.G. Diagnosis of urinary tract infection in children: fresh urine microscopy or culture? *Lancet* 338:767-770, 1991.
7. Waisman, Y., Zerem, E., Amir, L., and Mimouni, M. The validity of the uriscreen test for early detection of urinary tract infection in children. *Pediatrics* 104:E41, 1999.
8. Lohr, J.A., Portilla, M.G., Geuder, T.G., Dunn, M.L., and Dudley, S.M. Making a presumptive diagnosis of urinary tract infection by using a urinalysis performed in an on-site laboratory. *J. Pediatr.* 122:22-25, 1993.
9. Lockhart, G.R., Lewander, W.J., Cimini, D.M., Josephson, S.L., and Linakis, J.G. Use of urinary gram stain for detection of urinary tract infection in infants. *Ann. Emerg. Med.* 25:31-35, 1995.
10. Shaw, K.N., McGowan, K.L., Gorelick, M.H., and Schwartz, J.S. Screening for urinary tract infection in infants in the emergency department: which test is best? *Pediatrics* 101:E1, 1998.
11. Weinberg, A.G. and Gan, V.N. Urine screen for bacteriuria in symptomatic pediatric outpatients. *Pediatr. Infect. Dis. J.* 10:651-654, 1991.
12. Hoberman, A., Wald, E.R., Reynolds, E.A., Penschansky, L., and Charron, M. Is urine culture necessary to rule out urinary tract infection in young febrile children? *Pediatr. Infect. Dis. J.* 15:304-309, 1996.
13. Matthai, J., and Ramaswamy, M. Urinalysis in urinary tract infection. *Indian J. Pediatr.* 62:713-716, 1995.
14. Leanos-Miranda, A., Contreras-Hernandez, I., Camacho, R., Villagomez-Salcedo, E., and Cervantes-Gorayeb, I. Diagnostic yield of various urine tests in urinary tract infections. *Rev. Invest. Clin.* 48:117-123, 1996.