Paediatrica Indonesiana

VOLUME 49 July • 2009 NUMBER 4

Original Article

Diagnostic value of urine Gram staining for urinary tract infection in children

I Nyoman Sartika[1], Ketut Suarta[1], Pungky Ardhani[2]

Abstract

Background Urine culture is the standard procedure for diagnosis of urinary tract infection (UTI). To reduce the cost and time spent in examining cultures, several rapid methods have been developed including microscopic examination, chemical tests, and automated systems.

Objective To determine the value of microscopic examination of a Gram stained uncentrifuged drop of urine for diagnosis of UTI.

Methods We carried out a diagnostic test in Sanglah Hospital, Denpasar, from January 2007 to December 2007. Midstream urine specimens were Gram stained and then examined using a high power oil immersion lens. Twenty fields of view were examined and finding of two or more microorganisms was taken as positive result. Urine was also cultured and a yield of more than 10^5 CFU/ml was taken as positive result. The sensitivity, specificity, and predictive values of Gram stained urine were determined using urine culture as gold standard.

Results From 43 subjects in our study, 12 subjects (28%) had positive urine culture. The prevalence was higher in males (58%) than females. UTI was most commonly caused by *Klebsiella pneumoniae* (33%). The sensitivity, specificity, positive predictive value, and negative predictive valuey, of the urine Gram stain were 67% (95%CI 40 to 93), 9.7% (95%CI 91 to 100), 89%, 88%, respectively. The accuracy, positive likelihood ratio,negative likelihood ratio, and post-test probability of the urine Gram stain were 88, 21, 0.3, and 89%, respectively.

Conclusion Microscopic examination of Gram stained uncentrifuged drop of urine is specific and accurate, but not sensitive for the diagnosis of UTI. **[Paediatr Indones. 2009;49:205-8]**.

Keywords: *Gram stain, urinary tract infection, uncentrifuged drop of urine*

Urinary tract infection (UTI) is a common problem in children.[1-3] Although the majority of children with UTI have an excellent prognosis, there is a risk of serious complications in some cases, especially in those with obstructive malformation of the urinary tract or vesico-ureteric reflux (VUR). Children with VUR are potentially at risk of developing renal scarring if infection occurs; higher infection rates result in a greater possibility of scar formation.[4,5]

UTI in infants is difficult to be diagnosed on the basis of symptoms and signs. Quantitative urine culture is considered to be the standard procedure for adequate diagnosis.[4,6] In an attempt to reduce the cost and time expended in examining negative cultures, various methods have been devised to detect bacteriuria in urine specimens that have been sent for microbiological analysis. These include conventional and semi-automated methods such as estimation of pus cells, Gram staining of urine, fluorescent staining, and

From the Department of Child Health, Medical School, Udayana University, Sanglah Hospital, Bali, Indonesia (INS, KS).[1] From the Department of Child Health, Medical School, Gadjah Mada University, Dr. Sardjito Hospital, Yogyakarta, Indonesia (PA).[2]

Reprint request to: I Nyoman Sartika, MD**,** Department of Child Health, Medical School, Udayana University, Sanglah Hospital, Jl. Pulau Nias, Denpasar, Bali, Indonesia. Tel. +62-361-244038. Fax. +62-361-244038. E-mail: *sartikapur2003@yahoo.com*

detection of urinary substances including lactoferrin and urinary nitrite, as well as automated techniques like the Vitek Auto Microbic System.[7,8] The simplest, cheapest, and most reliable method for urine specimen evaluation is detection of bacteriuria using Gram stain examination of an uncentrifuged drop of urine. This method, which only requires a microscope and Gram staining reagents, can prove to be valuable in peripheral laboratories that do not have adequate resources for more complicated UTI diagnosis. Our study aimed to determine the diagnostic value of Gram stained examination of uncentrifuged urine for UTI diagnosis.

Methods

A diagnostic test study was conducted in the pediatric ward at Sanglah Hospital, Denpasar, Indonesia from January 2007 to December 2007. The study was approved by Ethics Committee for Research and Development of Sanglah Hospital, Denpasar.

The required number of subjects determined using the sample size formula for a diagnostic test was 43. We included subjects who came to the hospital with one or more symptoms including unknown fever, dysuria, polakisuria, urgency, and fever with costovertebral pain. Subjects who were less than three years of age, took antibiotics before the study, and whose parents refused to participate in the study, were excluded.

All urine specimens were collected using the midstream void technique. Urine specimens were homogenized, and a nickel-chrome loop, calibrated to 10 μL was used to take a drop of urine and apply it to a glass slide. The drop was allowed to air dry at room temperature without spreading. After the slides had dried, the samples were examined using the Gram stain. Microscopic examination was carried out with a 100x oil immersion objective lens and 20 fields of view were examined per slide. Observation of two or more microorganisms per high power oil immersion lens was taken as a positive reading.

Urine culture was used as a reference method and samples were cultured within two hours of submission. Culture was performed by using Uristrip filter paper strips, then applying 2 μL of urine onto plates of cystine lactose electrolyte deficient agar (CLED). Culture plates were incubated for 24-48 hours at 37°C. Cultures that yielded more than 10^5 CFU/mL were taken as positive.

The examiners of Gram stain and culture were different and both sets of examiners were blind to the results of the other method. The results of microscopic evaluation were compared with the culture results for each specimen. Sensitivity, specificity, positive, and negative predictive values were calculated.

Results

During our one year study period, 60 potential subjects suspected of having UTI were identified. We analyzed the urine of a total of 43 subjects using both the Gram stain and the urine culture.

From the 43 specimens examined, 26 (60%) were from male subjects and 17 (40%) were from female subjects. Characteristics of the study subjects are shown in **Table 1**. The incidence of positive cultures, as determined using the culture technique, was 28% (12 from 43 specimens). The isolated microorganisms were *Klebsiella pneumonia* (n = 4), *Enterobacter cloacae* (n = 3), *E. coli* (n = 2), *Staphylococcus aureus* (n = 1), *Proteus mirabilis* (n = 1), and *Enterobacter sakazaki* (n = 1).

Table 2 shows the results of urine testing obtained by Gram staining and urine culture. The correlation between results obtained by Gram stain and urine culture was evaluated in terms of sensitivity (67% [95% CI 40 to 93]), specificity (97% [95% CI 91 to 100]), positive predictive value (89%), and negative predictive value (88%).

Table 1. Baseline characteristics of the study subjects

Variable	Positive UTI culture n	Negative UTI culture n
Sex		
Males	7	19
Females	5	12
Age		
< 5 years	3	19
5-10 years	9	11
>10 years	0	1
Symptoms		
Fever	9	28
Costovertebral pain	7	12
Polakisuria	3	5
Dysuria	4	2
Urgency	2	2

Table 2. Bacteriuria detection using Gram staining and urine culture

Gram staining	Urine culture		Total
	Positive	Negative	
Positive	8	1	9
Negative	4	30	34
Total	12	31	43

Discussion

UTI is a common disease in children. These bacterial urinary infections are potentially dangerous not only because they may present as life threatening episodes with serious prognosis, but also because they may be the forerunners of severe renal disease of adulthood. Therefore early diagnosis and treatment are essential.[1,9,10]

Although urine culture is the standard diagnostic method, clinical symptoms, macroscopic and microscopic observation can be used as a basis for an initial diagnosis. In this study we evaluated the urine Gram stain for rapid diagnosis of UTI. Using urine culture as the gold standard, we showed that the urine Gram stain method had sensitivity of 67%, specificity of 97%, positive predictive value of 89%, negative predictive value 88%, accuracy of 88, positive likelihood ratio of 21, negative likelihood ratio of 0.3, and post test probability of 89%.

Gardezi[13] showed that Gram staining of an uncentrifuged drop of urine had sensitivity of 87%, specificity of 62%, positive predictive value of 58%, and negative predictive value of 91%. However in that study, the Gram stain was taken as being positive if observation of one or more microorganisms per high power oil immersion lens was taken in 30 fields.

Another study showed that if observation of one or more microorganisms per high power oil immersion lens had a sensitivity and specificity of about 90%, specificity could increase to almost 99% if observation of five or more microorganisms was taken as a positive result per high power oil immersion lens.[10] A systematic review conducted by Gorelick *et al.*[16] showed that Gram staining of urine had sensitivity of 93%, specificity of 95%,positive likelihood ratio of 18.5 and negative likelihood ratio of 0.07.

Inconsistent Gram staining results may be caused by differences in the Gram staining protocol used, as well as the criteria used to decide if the staining result is positive i.e. how many microorganisms must be observed and how many fields of view are examined. Our study is the same with study by Cardoso *et al*,[15] but the results are different. This may be because of differences in sample size. The sample size in our study was minimal.

The main advantage of performing microscopic examination of Gram stained urine is rapid diagnosis of urinary infection to give guidance for initial patient treatment. Microscopic examination of an uncentrifuged Gram stained urine drop is the conventional microscopic method for diagnosing urine specimens with counts of 10^5 CFU/mL. It was very easy and less time consuming to have two or more bacteria per field. Observation of Gram stained urine also helped us to predict the type of bacteria causing the infection; this can be confirmed by culturing the urine.

Health centers and laboratories with poor resources usually lack of appropriate methods for evaluation of urine specimens. In such areas, simple Gram staining can be an effective method for ruling out the possibility of UTI, thus saving time and money.[3,13] In our study, it was observed that post test probability of Gram staining was 89%; this means that if there is positive result for Gram staining we do not have to complete urine culture, and that the therapy can be started immediately.

In conclusion, microscopic examination of a Gram stained uncentrifuged drop of urine is specific and accurate, but not sensitive for diagnosis of urinary tract infections.

Acknowledgments

My highest gratitude to I Gde Raka Widiana, MD, and IB Subanada, MD for their help in constructing the methodology and statistical analysis used in this study.

References

1. Gulati S, Kher V. Urinary Tract Infection. Indian Pediatr. 1996;33:211-7.
2. Larcombe J. Urinary tract infection in children. BMJ. 1999; 319:1173-5.

3. Dhakal BK, Pokhre BM, Ahnn J. Microscopic detection of urinary tract infection in Nepalese patients. J Microbiol 2002. 40:267-73.
4. Tambunan T, Madiyono B, Sihombing R. Vesico-ureteric reflux with and without reflux nephrophaty in children with urinary tract infection. Paediatr Indones. 2002; 42:125-9.
5. Zorc JJ, Kiddoo DA, Shaw KN. Diagnosis and management of pediatric urinary tract infections. Clin Microbiol Rev. 2005; 18:417–22.
6. Hansson S. Urinary tract infection. In: Barratt TM, Avner ED, Harmon WE, editors. Pediatric nephrology. 4th edition. Philadelphia: Williams & Wilkins, 1999; p. 835-47.
7. Okada H, Sakai Y, Miyazaki S, Arakawa S, Hamaguchi Y, Kamidono S. Detection of significant bacteriuria by automated urinalysis using flow cytometry. J Clin Microbiol. 2000; 38:2870-2.
8. Tilton RE, Tilton RC. Automated direct antimicrobial susceptibility testing of microscopically screened urine cultures. J Clin Microbiol. 1980; 11:157-61.
9. Tokan HB, Kari K. Clinical aspects and white blood cell count in children with urinary tract infection. Paediatr Indones. 1999; 39:38-46.
10. WHO. Urinary Tract Infections in Infants and Children in Developing Countries in the Context of IMCI [cited 2008 March 24th]. Available from: URL: http://whqlibdoc.who.int/hq/2005/WHO_FCH_CAH_05.11.pdf
11. Garcia FJ, Nager AL. Jaundice as an early diagnostic sign of urinary tract infection in infancy. Pediatrics. 2002; 109:846-51.
12. Kontiokari T, Nuutinen M, Uhari M. Dietary factors affecting susceptibility to urinary tract infection. Pediatr Nephrol. 2004; 19:378-83.
13. Gardezi A, Mirza SH, Khursheed U, Farooque M, Waqar A. Microscopy of Gram stained uncentrifuged drop of urine for presumptive diagnosis of urinary tract infections. Pak J Pathol. 2006; 17(3):111-4.
14. Pezzlo MT, Tan GL, Peterson EM, Luis M. Screening of urine cultures by three automated systems. J Clin Microbiol. 1982; 15:468-74.
15. Cardoso CL, Muraro CB, Siqueira VLD, Guilhermetti M. Simplified technique for detection of significant bacteriuria by mikroscopic examination of urine. J Clin Microbiol. 1998; 36:820-3.
16. Gorelick MH, Shaw KN. Screening tests for urinary tract infection in children: a meta-analysis. Pediatrics. 1999; 104:1-7.