THE VALUE OF POSITIVE NITRITES IN SCREENING ASYMPTOMATIC BACTERIURIA AMONGST MALAYSIAN SCHOOL CHILDREN

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Abstract. It is important to diagnose and treat urinary tract infection in children before renal damage has taken place. Hence a new screening procedure will be of interest.

This study was conducted to evaluate the efficacy of urinary nitrite in screening for asymptomatic bacteriuria among school children compared to a more traditional method.

Of the 44,816 school children investigated 240 (0.54%) students were judged to have bacteriuria *ie* 82 (0.19%) in boys and 158 (0.35%) in girls. *Escherichia coli* was the commonest organism isolated (28.75%). Urine dipstick testing for nitrite was found to have a low sensitivity and positive predictive value. While urinalysis for pyuria was noted to have a sensitivity of 77.9%, a specificity of 95.8% and a negative predictive value of 99.9%.

INTRODUCTION

Urinary tract infection (UTI) in children is frequently more difficult to diagnose than that in adults (Hamoudi *et al*, 1986). Forty percent of infections in children either asymptomatic or symptomatic are not directly referable to the urinary tract (McCracker, 1987). The gold standard to confirm urinary tract infection is urine culture. However this is time consuming and laborious for routine screening.

A rapid method is helpful, *ie* dipstick method. However its reliability, sensitivity and specificity are usually the questions posed. Previous reports have found that dipstick testing to be useful in predicting the outcome of urine culture (Smalley and Dittman, 1983; Wenk *et al*, 1982; Wise *et al*, 1983), while others conclude that dipstick testing (leukocyte esterase nitrite strip) was neither sensitive nor specific enough to be used as a cost effective method for screening out negative urine (Wilkins *et al*, 1985).

In our community we have yet to report the efficacy of this method in comparison with a more traditional method such as microscopic examination for pyuria which requires the expertise of a trained technologist. This study is meant to assess the value of urinary nitrites in screening for asymptomatic bacteriuria amongst Malaysian school children.

MATERIALS AND METHODS

Primary school children from three districts in Kelantan Malaysia *ie* Tumpat, Bachok and Kota Bharu were directly questioned at their respective schools to ensure that they did not have symptoms of UTI. Only those with no symptoms suggestive of UTI were included into this study.

A total of 44,816 primary school children *ie* 23,132 boys and 21,684 girls were enrolled into the study. Their age ranged from 7-12 years.

Mid-stream urine collection was explained to students. Three urine samples were collected in sterile bottles. One of the urine samples collected were examined using a dipstick strip Medi-Test Combi 4A for nitrite while the second urine samples were examined microscopically for pyuria and bacteria. The third urine sample was immediately sent for culture.

Dipstick testing for nitrite

A reagent strip as mentioned above was dipped for aproximately 1 second into fresh urine. It was then withdrawn over the rim of the container to

remove excess urine. After 30-60 seconds the color on the test strip was compared with the color scale provided. The principle of Griess reaction is the basis of this study.

Microscopic examination for pyuria and bacteria

10 ml of urine were placed in a plastic centrifuge tube. Urine was centrifuged for 5 minutes at 1,000*g*. The sediments were resuspended in 0.5 ml of supernatant. One drop of sediment suspension was placed on a glass slide, coverslipped and examined microscopically for formed element and bacteria. The leukocyte count was recorded (per high power field) and an estimate of bacteria was recorded as none, few, moderate or many. Significant pyuria was defined as the presence of 5 or more pus cells per high power field.

Urine culture

Two plates of cysteine-lactose electrolyte deficient (CLED) agar were immediately inoculated, one with 0.1 ml of undiluted urine and the other with 0.1 ml of 100-fold diluent. After overnight incubation at 35°C, colony forming units/ml were estimated by multiplying the number of colonies on the CLED agar by 1,000.

Urinary colony count of over 10^5 organisms/ml urine in pure culture was read as significant growth or positive. While urinary colony count of less than 10^5 organisms per ml was read as insignificant growth (negative).

Urine cultures which grew mixed organisms were also considered as a negative culture.

Statistical analysis

The equations below were used to calculate sensitivity, specificity and positive and negative predictive value (Galen and Peters, 1987).

$$\text{Sensitivity} = \frac{\text{No. of true positives}}{\text{No. of true positives + false negatives}}$$

$$\text{Specificity} = \frac{\text{No. of true negatives}}{\text{No. of true negatives + false positives}}$$

$$\text{Positive predictive value} = \frac{\text{No. of true positives}}{\text{No. of true positives + false positives}}$$

$$\text{Negative predictive value} = \frac{\text{No. of true negatives}}{\text{No. of true negatives + false negatives}}$$

RESULTS

Positive cultures were detected in 240 (0.54%) school children: 82 (0.19%) of them were boys and 158 (0.35%) were girls. *Escherichia coli* was the most common organism isolated (28.75%) as shown in Table 1.

Of the 44,816 urine specimens tested, 3,988 had mixed growth and 1,039 had growth of organisms less than 10^5 per ml. Both of which were considered as negative culture. The relationship of dipstick testing for nitrites, microscopic examination for pyuria and bacteria to urine culture was analysed and results are shown in Table 2.

A high false negative result with nitrite testing was noted while microscopic examination for pyuria had a high false positive result.

The sensitivity, specificity and positive and negative predictive values of each particular test were calculated as shown in Tables 3, 4, 5.

The sensitivity of urinary nitrites in screening for asymptomatic bacteriuria was 8.3%. This was far lower than urinalysis study for pyuria and bacteriuria. Similarly the positive predictive values of urinary nitrites was found to be lower than that of microscopic examination for bacteriuria.

Table 1

Organisms isolated at school screening.

	Organisms	n = 240	%
1	*Escherichia coli*	69	28.75
2	*Staphylococcus* sp	61	25.42
3	*Klebsiella* sp	45	18.75
4	*Streptococcus* sp	39	16.25
5	*Proteus* sp	15	6.25
6	Others	11	4.58

Table 2

Comparison of nitrite and microscopic urine analysis with urine culture.

Dipstick and microscopic analysis	Urine culture	
	Positive	Negative
Nitrite		
No. positive	20	34
No. negative	220	44,542
Microscopic examination for pyuria		
No. with > 5 wbc/hpf	187	1,873
No. with < 5 wbc/hpf	53	42,703
Microscopic examination for bacteria		
No. with moderate or many bacteria	177	54
No. with none of few bacteria	63	44,522

Table 3

Sensitivity, specificity and predictive values of the dipstick testing for nitrite.

Nitrite	Calculation × 100	Index (%)
Sensitivity	20/(20 + 220)	8.3
Specificity	44,542/(34 + 44,542)	99.9
Positive predictive value	20/(20 + 34)	37.0
Negative predictive value	44,542/(44,542 + 220)	99.5

Table 4

Sensitivity, specificity and predictive values of microscopic examination for pyuria.

Pyuria	Calculation × 100	Index (%)
Sensitivity	187/(187 + 53)	77.9
Specificity	42,703/(42,703 + 1,873)	95.8
Positive predictive value	187/(187 + 1,873)	9.1
Negative predictive value	42,703/(42,703 + 53)	99.9

Table 5

Sensitivity, specificity and predictive values of urine microscopic examination for bacteria.

Characteristic	Calculation × 100	Index (%)
Sensitivity	177/(177 + 63)	73.8
Specificity	44,522/(44,522 + 54)	99.9
Positive predictive value	177/(177 + 54)	76.6
Negative predictive value	44,522/(44,522 + 63)	99.9

DISCUSSION

It is of importance to detect asymptomatic bacteriuria amongst children, in order to give treatment before renal damage has taken place. Hence new useful screening procedures for bacteriuria detection are of interest.

The present paper deals with a high number of asymptomatic Malaysian school children screened for bacteriuria by means of a nitrite dipstick, microscopy and urine culture.

Of the 44,816 school-children investigated 240 children were judged to have bacteriuria, according to the chosen criteria. These criteria are commonly used, but their relevance are highly discussed (Bolann *et al*, 1989). In 3,988 cases mixed growth was detected and in 1,039 cases bacterial growth occurred but at less than 100,000 bacterial per ml. It is a matter of philosophy how many of the latter two groups really also may have had an infection in their urinary tract. The present numbers of asymptomatic bacteriuria found are low compared to some other investigators' findings (Kunin *et al*, 1962; Dodge *et al*, 1969; Iitaka *et al*, 1990).

The test for nitrite depends on several factors:

a) the nitrite intake must be sufficiently high for urinary nitrate excretion. Here local food intake may be of importance (vegetables contain much nitrate).

b) the bacteria present must be able to form nitrite from excreted nitrate (most gram negative bacteria do so).

c) time for bacterial growth and nitrite formation in the bladder must be sufficiently long (morning specimens may be preferable).

d) there must not be too much excretion of inhibitors for the test strip reaction (*eg* ascorbic acid may cause a false negative nitrite test).

If the above mentioned criteria are not fulfilled, the nitrite test frequently becomes false negative. The exact cause of the low sensitivity result of our nitrite testing compared to previous reports (Loo *et al*, 1986; Christenson *et al*, 1985; Morrison and Lum, 1986) is difficult to establish. Perhaps the food consumed by our children contained less nitrate compared to children participating in earlier studies. If this is true, then there is a need to emphasise on this alimentary factor in order to improve the sensitivity of this test in our community.

If positive, however urinary nitrite has been found to have a high positive predictive value for real bacteriuria when the prevalence for UTI has been moderate to high (Bolann *et al*, 1989). For this reason the test has been included in most urinary screening in hospitals around the world today.

The relative low positive predictive value for nitrite test found in this study is not easily explained but may be partly due to the very few true positives found via the chosen criteria for real bacteriuria and partly by a generally existing low prevalence of UTI in school children. Perhaps the combination of a chemical nitrite and leukocyte esterase test should be used in order to improve the positive predictive value as shown in earlier study (Bolann *et al*, 1989).

In conclusion, with a sensitivity of 8.3% and positive predictive value of 37% we do not recommend urinary dipstick testing for nitrite to be used in screening for asymptomatic bacteriuria in our community especially when more reliable methods are available.

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REFERENCES

Bolann BJ, Sandberg S, Digranes A. Implications of probability analysis for interpretating results of leukocyte esterase and nitrite test strips. *Clin Chem* 1989; 35 : 1663-68.

Christenson RH, Tucker JA, Allen E. Results of dipstick tests, visual inspection, microscopic examination of urine sediment and microbiological cultures of urine compared for simplifying urinalysis. *Clin Chem* 1985; 31 : 448-50.

Dodge WF, West EF, Fras PA, *et al.* Detection of bacteriuria in children. *J Paediatr* 1969; 74 : 107-10.

Galen RS, Peters T. Analytical goals and clinical relevance of laboratory procedures. In: Tietz NW, ed. Fundamental Clinical Chemistry. 3rd ed. Philadelphia: WB Saunder, 1987 : 213-24.

Hamoudi AC, Bubis SC, Thompson C. Can the cost savings eliminating urine microscopy in biochemically negative urines be extended to the pediatric population? *Am J Clin Pathol* 1986; 86 : 658-60.

Iitaka K, Tadasu S, Oyama K, Izawa T, Igarashi S. Screening for bacteriuria in Japanese school children. *Acta Paediatr Jpn* 1990; 32 : 690-5.

Kunin CM, Zacha E, Paquin AJ. Urinary tract infections in school children 1. Prevalence of bacteriuria and associated urologic findings. *N Engl J Med* 1962; 266 : 1287-96.

Loo SY, Scottolini AG, Luangphinith S, Adam AL. Performance of a urine-screening protocol. *Am J Clin Pathol* 1986; 85 : 479-84.

McCracken GH. Diagnosis and management of acute urinary tract infections in infants and children. *Paediatr Infect* 1987; 6 : 107-12.

Morrison MC, Lum G. Dipstick testing of urine - can it replace urine microscopy? *Am J Clin Pathol* 1986; 85 : 590-4.

Smalley DL, Dittman AN. Use of leukocyte esterase-nitrate activity as predictive assays of significant bacteriuria. *J Clin Microbiol* 1983; 18 : 1256-7.

Wenk RE, Dutta D, Rudert J, Kim Y, Steinhagen C. Sediment microscopy, nitrituria and leukocyte esterasuria as predictors of significant bacteriuria. *J Clin Lab Automat* 1982; 2 : 117-21.

Wise KA, Grammens GL, Sagert LA. The use of urinary leukocyte esterase and nitrite as an aid in predicting urine culture results. Abstract of the Annual Meeting of the American Society for Microbiology, 1983 : 313.

Wilkins EGL, Ratcliffe JG, Roberts C. Leucotyte esterase-nitrite screening method for pyuria and bacteriuria. *J Clin Pathol* 1985; 38 : 1342-5.