

Evaluation of Dipstrips, Direct Gram Stain and Pyuria as Screening Tests for the Detection of Bacteriuria

Pages with reference to book, From 38 To 41

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Abstract

Two hundred and fifty cases of clinically suspected urinary tract infection were analysed for the detection of bacteriuria. Parameters studied included direct Gram staining, pyuria on microscopic examination of uncentrifuged urine and a 'strip method for the detection of blood, protein, nitrite and leucocyte esterase. Significant bacteriuria (colony count 10^5 per ml) was found in 112 cases with a positivity ranging from 65 to 83% for the presence of food, protein, nitrite and leucocyte esterase. Highest positive predictive values were obtained with the presence of nitrite and leucocyte esterase (98%), blood, protein and nitrite (94%) as well as with blood, protein, nitrite and leucocyte esterase (98%). Both pyuria and direct Gram staining were positive in 85% cases. The combined presence of both these parameters gave 100% positive predictive value. Gram staining combined with pyuria was more effective and economical as compared to the dipstrips for the detection of bacteriuria (JPMA 46: 38, 1996).

Introduction

Urinary tract infection (UTI) cannot be diagnosed on clinical grounds alone¹. Confirmation of UTI requires documentation of bacteria in bladder urine². However, the culture in clinical practice is usually done on voided urine which in women is easily contaminated with perineal bacteria. Quantitative culture and specific identification of the organisms in urine are used to distinguish contamination from true urinary tract infection^{2,3}. Bacterial counts by conventional methods are difficult to adopt in an ordinary diagnostic laboratory and are expensive in time and material⁴⁻⁶. The simple methods like calibrated loops, blotting papers, strips and dipslides also require overnight incubation⁴⁻⁸. As an alternative to the conventional methods, several presumptive tests are available which either quantitate bacteria by non-cultural methods or suggest infection by the demonstration of an inflammatory reaction. Such tests are microscopy for pyuria and bacteriuria, chemical tests for detection of pus cells and bacteria in urine and automated methods for bacteriuria detection⁹⁻¹¹.

Pyuria is a term used to describe the appearance of increased number of polymorphonuclear leucocytes in the urine¹². It is an evidence of acute inflammation¹³. However, pyuria is not always associated with bacteriuria and its absence does not exclude infection^{14,15}. Leucocytes are normally absent in midstream urine from healthy children and male subjects, The urine of adult women frequently contains leucocytes and a small number should be regarded as normal⁹. Ten cells per cubic mm in uncentrifuged urine is the upper limit of normal leucocyte excretion¹². The presence of 100 pus cells per cubic mm in uncentrifuged urine specimens correlates well with infection^{9,12,16}.

Microscopy of urine for bacteriuria is a rapid screening technique which has been used for years as a standard laboratory practice¹¹. Urine can be examined stained or unstained and centrifuged or uncentrifuged. The most widely used method is the Gram stained smear of fresh uncentrifuged specimen of urine examined under oil immersion objective¹¹. Visualizing any bacteria by this technique is reported positive, with the sensitivity being 69 to 96%¹⁷⁻²⁰.

As Enterobacteriaceae, the most prevalent organisms found in infected urine, reduce nitrate to nitrite^{21,22}, so the nitrate reduction test is a useful tool and nitrite detection is an indicator of UTI²³. Presence of blood or protein in the urine detected by multipatch strips also indicates urinary tract infection^{24,25}. Sensitivity of more than 85% and negative predictive value of more than 86% has been reported^{24,26}. Pyuria can also be assessed with dipstrips. The esterase present in neutrophils are detected with the help of a dipstrip²⁷. This parameter with the presence of blood, protein and nitrite in urine gives a sensitivity from 91% to 94% and negative predictive value from 89% to 95%^{24,25}. The present study compares the screening of urine by Gram staining and pyuria assessment in uncentrifuged urine with the dipstrip tests for blood protein nitrite and leucocyte esterase.

Material and Methods

Two hundred and fifty specimens of urine were obtained from both patients and in-patients of Sir Ganga Ram Hospital and Mayo Hospital, Lahore from patients with suspected UTI irrespective of age and sex. Brief history of patients was obtained and patients on antibiotics were excluded. Urine samples from each patient were labelled and immediately transported to the laboratory where they were gently shaken and divided into two aliquotes. In case of delay, samples were kept at 4°C and analysed within two hours of collection. One aliquot was used for screening tests and the other for inoculating culture media (blood agar and MacConkey agar).

The dipstrip examination of the urine samples was carried out with Multistix 8 (Ames, Miles Inc. Diagnostic Division, Elkhart, In 46515, USA) urine strips. The strip was dipped in fresh uncentrifuged specimen of urine and read according to the manufacturers instructions.

Method of Collee et al²⁸ was used to determine the presence of pus cells in uncentrifuged urine.

Gram stained smears from uncentrifuged urine were made by spreading one p.l (0,001 ml) of urine on a 3x1 inch clean, grease free glass slide with a calibrated loop over an area of 3-5 mm diameter. Eight similar smears were made per slide and examined under oil immersion objective. Presence of Gram negative rods in any high power field constituted a positive smear for infection.

Results

Among the 250 cases, there were 112 clinically significant cultures, the chemistry of which is given in Table 1.

Table I. Status of urinary chemistry in clinically significant culture positive UTI cases.

	Positive for blood	Positive for protein	Positive for nitrate	Positive for leucocyte esterase
Culture Positive (112)	<u>76 (68)</u>	73 (65)	77 (69)	93 (83)
Culture Negative (138)	36 (26)	39 (28)	35 (25)	19 (14)

Figures in parentheses indicate percentage.

The comparison of clinically significant culture with blood, protein, nitrite and leucocyte esterase in urine, singly as well as in combination is shown in Table II.

Table II. Comparison of clinically significant culture with blood, protein, nitrite and leucocyte esterase in urine.

Group	Sensitivity	Specificity	Positive Predictive value	Negative Predictive Value
	%	%	%	%
Blood	68	76	70	74
Protein	65	61	57	68
Nitrite	69	83	76	76
Leucocyte esterase	84	87	83	86
Blood and protein	80	76	73	83
Blood and nitrite	76	96	93	84
Nitrite and protein	74	83	79	78
Nitrite and leucocyte esterase	90	99	98	94
Blood, protein and nitrite	86	95	94	90
Blood, protein, nitrite and leucocyte esterase	96	98	98	97

The presence of blood, protein, nitrite and leucocyte esterase were found to be significant individually and in combination, in detecting infected urine samples. They also provided a high sensitivity and specificity as well as positive and negative predictive values..

The comparison of clinically significant culture with Gram staining of uncentrifuged urine and with pyuria, was also found to be significant in detecting infected urine samples (Tables III and IV).

Table III. Comparison of clinically significant culture with Gram staining of uncentrifuged urine.

Group	Culture Positive	Culture Negative	Total
Gram stain*			
Positive	95	0	95
Gram stain Negative	17	138	155
Total	112	138	250

*P<0.001 as compared with Gram stain negative

Sensitivity	85%
Specificity	100%
Predictive value for:	
Positive result	100%
Negative result	88%

Table IV. Comparison of clinically significant culture with pyuria.

Group	Culture Positive	Culture Negative	Total
Pus cells*			
Positive	95	3	98
Pus cells Negative	17	135	152
Total	112	138	250

*P<0.001 as compared with pus cells negative.

Sensitivity 85%

Specificity 98%

Predictive value for:

Positive result 97%

Negative result 89%

Sensitivity of more than 84% was found with both these parameters while positive predictive value with Gram staining was 100% and with pyuria assessment 97%.

Discussion

The present study supports the view that presence of blood, protein and nitrite and leucocyte esterase in urine are good indicators of urinary tract infection²⁴⁻²⁶. High positive predictive values obtained in the present study could be due to collection of samples from symptomatic patients not taking any antibiotics. The study samples were either processed promptly or were kept at 4°C and processed within two hours of collection. Other workers examined random urine samples including catheter, midstream, clean catch and bag specimens, irrespective of time of collection, storage or transportation conditions¹².

In this study the nitrite test was positive in significant numbers of culture positive cases. Varying results have been reported by other workers. Low sensitivity of nitrite test (39%) was reported by Moffat et al⁵, whereas Sinaniotis et al²⁹ found nitrite test unable to detect 40% of cases with proven bacteriuria. Craig et al⁸, observed 100% sensitivity and specificity in a field study and 42% positivity in hospital cases with significant bacteriuria.

Most common organisms responsible for urinary tract infection reduce nitrate to nitrite²¹. Consequently the presence of non-nitrate reducing organisms in urine can give false-negative results. In the present study, non-nitrate reducing organisms were present in 12% of the cases. Craig et al⁸ found that positive nitrite test strongly suggests UTI when done on first morning sample which allows a long period for nitrite to accumulate in the bladder. The test was not found useful and gave false negative results on randomly collected samples, or for patients receiving parenteral nutrition without a nitrogen source

when concentration of bacteria was less than 10^5 per ml or when urine was diluted or slow growing organisms were present^{8,30,31}. James et al²³ showed urobilinogen to interfere with nitrite analysis and the test was not reliable in the presence of elevated levels of urobilinogen. Factors such as phenzopyridine, blood, ascofoic acid and bilirubin also influence the sensitivity of the test²².

The sensitivity of leucocyte esterase in detecting infected urine samples reported by Marquette et al³² was 83% and by Roberston and Duff³¹ 77%. However, the specificity was 70% and 96% respectively. This test is based on esterases present in polymorphonuclear cells which convert indoxyl cathomc ester, the substrate of dipstrip, to indoxyl and this in the presence of atmospheric oxygen becomes indigo blue³³. The esterase activity is not influenced by bacteria, commonly used drugs or variable composition of urine and is not dependant on intact cells³⁴, Trichomonas can give a positive leucocyte esterase reaction³⁵. Two samples of urine obtained from female patients in the present study gave a positive leucocyte esterase test in absence of pyuria due to trichomonas infection.

The combined strip tests when compared with culture positive cases gave comparable results. However, lower specificity and positive predictive value were reported as compared to the present study²⁴.

The presence of pus cells in urine is an evidence of acute inflammation¹³. However, pyuria without bacteriuria can occur in tuberculosis, urinary calculi, toxic nephropathy, bladder tumors, resolving bacterial infections, bacterial infections with antimicrobial agents in urine, infection adjacent to the urinary tract and abacterial infections³⁶. In this study pus cells were found in 85% of culture positive and 2% of culture negative cases. While 15% of infected cases were negative for pus cells. The overall sensitivity of this indicator for infection was 85% and specificity was 98%. The predictive value for positive result was 97% and for negative result 89%. Results reported by various workers³⁷⁻³⁹. vary depending upon the patient population and the presence of complicating factors such as diabetes, calculus or analgesic nephropathy.

The Gram staining of uncentrifuged urine was found to be significant ($P < 0.001$) in detecting culture positive cases in the present study. Sensitivity was 75% when compared with total culture positive cases and 85% with clinically significant cultures. The results are comparable to those reported by other workers^{17,19,20}. where sensitivity varied from 89% to 93%, whereas Jorgenson and Jones¹⁸ detected 69% of all urine samples by Gram staining and 74% of those containing Gram negative bacilli. The chemical tests by dipstrips were introduced as cost effective measures^{20,33}, but in our country the strips are costly and uneconomical. As technical staff is easily available and the volume of work is large, it is more feasible to examine the uncentrifuged urine for pus cells. This alongwith Gram staining are strong indicators of infection and have high positive predictive values. Both techniques have proved to be effective and inexpensive for diagnosing urinary tract infection.

Acknowledgements

The help of Dr. Taj Bano, Dr. Zaibunmsa and others at Mayo and Sir Ganga Ram Hospital in conducting this study and Mr. Abdul Ghaffar Naeem for typing the manuscript is gratefully acknowledged.

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