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IMPORTANCE OF LEUCOCYTE ESTERASE TEST IN DIAGNOSIS OF URINARY TRACT INFECTION

***[1]PRIYANKA YADAV (MBBS), [2]APARNA (MBBS, MD), MADHU SHARMA (MBBS, MD) AND UMA CHAUDHARY (MBBS, MD)**

[1]Postgraduate Student Department of Microbiology Pt. B D Sharma, PGIMS Rohtak
[2]Professor Department of Microbiology Pt. B D Sharma, PGIMS Rohtak
[3]Professor Department of Microbiology Pt. B D Sharma, PGIMS Rohtak
[4]Senior Professor & Head Department of Microbiology Pt. B D Sharma, PGIMS Rohtak

ABSTRACT

Introduction: Hospital diagnostic laboratories investigate many thousands of urine specimens each year, but only a small proportion(about 25-30%) of it are infected. Lot of staff time and culture material are spent on specimens that yield insignificant growth. Therefore, a simple inexpensive test is needed as a preliminary test in routine practice to select those urine samples only which require further examination in the laboratory. Leucocyte esterase test has the advantage of detecting esterases in both intact and lysed leucocytes. Therefore, even specimens that have not been preserved properly will yield a positive test result. Aims & objective: The present study was designed to compare the reliability of wet mount examination and leucocyte esterase activity with that of a urine culture in diagnosing bacteriuria and pyuria in patients of UTIs. Material and Methods: This study was conducted on a total of 600 urine samples received in the Microbiology department, Pt BDS PGIMS, Rohtak. The samples were processed as per standard procedures and subjected to microscopic examination, dipstick testing, TTC test and culture within 30 minutes of sample collection. Result: Of 600 samples 144 were LET positive, of which 106 were positive by urine culture and 38 were urine culture negative. Similarly out of 600 samples 456 were LET negative, of which 417 were negative by urine culture while 39 were positive by urine culture. Out of the 145 culture positives, *E. coli* (44.1%) was most common pathogen. Conclusion: Rapid dipsticks tests for LET are reliable alternative to culture for screening of UTIs.

KEY WORDS: UTI, LET, bacteriuria, pyuria

PRIYANKA YADAV
Postgraduate Student Department of Microbiology
Pt. B D Sharma, PGIMS Rohtak

***Corresponding author**

INTRODUCTION

UTI is a common and painful human illness that, fortunately, is rapidly responsive to modern antibiotic therapy. In the preantibiotic era, UTI caused significant morbidity. UTI affects both male and female of all age groups. However, it is particularly common in females, about 10-20% of women have UTI at some time in their life and significant numbers have recurrent infection.[1] UTIs are defined as significant bacteriuria in the presence of clinical signs and symptoms. *Escherichia coli* is by far the commonest cause of uncomplicated UTIs. Other bacteria frequently isolated from patients with UTIs include *Klebsiella* spp*., Proteus* spp*., Enterobacter* spp.*,*other members of family *Enterobacteriaceae*, *Staphylococcus saprophyticus,* and enterococci. In complicated cases of UTIs, particularly in recurrent infections, the relative frequency of isolation of *Proteus* spp*., Pseudomonas* spp*., Klebsiella* spp.and *Enterobacter* spp. increases.[2]UTI is of two types, symptomatic or asymptomatic. Asymptomatic bacteriuria (ASB) or asymptomatic UTI is defined as the presence of a significant quantity of bacteria in a properly collected urine specimen from a person without symptoms or signs of UTI.[3,4]Significant bacteriuria is defined as presence of colony count of $>10^5$ cfu/ml of a single species in a mid stream clean catch sample.[5]"Pyuria" is the presence of increased numbers of polymorphonuclear leucocytes in the urine and is evidence of an inflammatory response in the urinary tract.[6] Although pyuria is the most prevalent manifestation of UTI; it can also occur in other important conditions such as pregnancy, fever and administration of corticosteroids.[7] The continuing emphasis on cost effectiveness and the increasing number of requests for the examination of urine has produced several rapid laboratory screening methods to help eliminate unnecessary examination by culture.[8]Advantage of using rapid tests is that rapid diagnostic tests can rule out negative samples, are economical and save valuable time.[9] Many rapid diagnostic tests are available which include wet mount microscopy, Gram's stain, dipstick and automated assays.[10]Leucocyte esterase test is based on the hydrolysis of ester substrates by proteins with esterolytic activity. Human neutrophils produce as many as 10 proteins with esterolytic activity. These proteins react with ester substrates to produce alcohols and acids which react with the chemicals to produce a colour change that is proportional to the amount of esterase in the specimen. This test has the advantage of detecting esterases in both intact and lysed leucocytes. Therefore, even specimens that have not been preserved properly will yield a positive test result.[11]

AIMS AND OBJECTIVES

The purpose of this investigational study was to compare the reliability of wet mount examination and leucocyte esterase activity with that of a urine culture in diagnosing bacteriuria and pyuria in patients of UTIs.

MATERIALS AND METHODS

This study was conducted on a total of 600 urine samples of both indoor and outdoor patients irrespective of the age and sex differences. All the urine samples received in the Bacteriology Laboratory were processed as per the standard guidelines. About 15-20 mL of clean catch mid stream urine samples were collected in sterile container (or sterile test tube) and subjected to microscopic examination, dipstick testing, TTC test and culture within 30 minutes of sample collection. The presence of one pus cell per seven high power fields (corresponding to 10^4 leucocytes per ml) and numbers higher than this indicated significant pyuria. All the samples were grouped according to the number of pus cells per high power field and each group was compared with semi-quantitative culture.[12] Dipstick leucocyte esterase test (LET) was evaluated using Combur[10]Test M Cobas test strips (Roche Diagnostics, India). Manufacturer's methodology was followed to perform the tests and results were interpreted accordingly.[3]The test strips were dipped in the urine sample and taken out immediately (maximum 1 sec). While withdrawing the strip, its edge was wiped along the edge of test tube to remove excess urine. Visual reading was taken by comparing the colours after 60-120 sec for LET. Colour change was seen in positive samples. In a positive LET, colour changed from colourless to pink (+1), light purple (+2) and dark purple (+3). Culture was done on blood agar and

MacConkey agar, all the plates were incubated at 37^0C and inoculated media were examined for the growth after overnight incubation. The evaluation of colony morphology on the plating media were done and the subsequent identification procedures were carried on the isolated bacteria, using standard procedures.[13]

RESULTS

During the study period of 1 year, a total of 600 patients were selected randomly. Out of 600 patients, 265 (44.2%) were males and 335 (55.8%) were females. Out of the total patients, majority 179(29.8%) were in the reproductive age group 21-30 years, followed by 89 (14.8%) in the age group 41-50 years. Out of total 600 patients majority 149 (24.8%) were from urology department followed by department of gynae/obstetrics 146 (24.3%) and surgery 61(10.2%).Out of the total 600 samples 200 were microscopy positive, of which 121 were positive by culture and 79 were culture negative. Similarly out of 600 samples 400 were negative for microscopy of which 376 were negative by urine culture while 24 were positive by urine culture. Comparing microscopy with urine culture sensitivity, specificity, PPV and NPV were 83.4%, 82.6%, 60.5% and 94.0% respectively (Table 11)Table 11 shows that out of 600 samples 144 were LET positive, of which 106 were positive by urine culture and 38 were urine culture negative. Similarly out of 600 samples 456 were LET negative, of which 417 were negative by urine culture while 39 were positive by urine culture. Comparing LET with urine culture sensitivity, specificity, PPV and NPV were 73.1%, 91.6%, 73.6% and 91.4% respectively.On culture, out of 600 samples 428 (71.3%) were sterile, 27 (4.5%) showed colony count $<10^5$ cfu/ml (Non-Significant Bacteriuria) and 145 (24.17%) had significant growth with colony count $>10^5$ cfu/ml. Out of the total 145 patients with positive urine culture, *E. coli* was most common and was seen in 64 patients (44.1%), *Klebsiella* spp was seen in 19 patients (13.1%), *Pseudomonas aeruginosa* was seen in 16 patients (11.1%), CONS was seen in 13 patients (8.9%), *S. aureus* was seen in 10 patients (6.9%), *Enterobacter* spp was seen in 9 patients (6.2%), *Enterococcus* spp was seen in 7 patients (4.8%), *Proteus* spp was seen in 5 patients (3.5%) and *Citrobacter* spp was least common & seen in 2 patients (1.4%).

Table 1
Comparison of wet mount microscopy with culture

MICROSCOPY	Culture		
	Negative	Positive	Total
Negative	376	24	400
Positive	79	121	200
Total	455	145	600

Sensitivity: 83.4%; Specificity: 82.6%; PPV: 60.5%; NPV: 94.0%

Table 11
Comparison of LET with Culture

LET	Culture		
	Negative	Positive	Total
Negative	417	39	456
Positive	38	106	144
Total	455	145	600

Sensitivity: 73.1%; Specificity: 91.6%; PPV: 73.6%; NPV: 91.4%

DISCUSSION

Urinary Tract Infections (UTIs) are the second most common infections next to respiratory tract infections causing significant morbidity. UTIs account for more than 8 million outdoor patient visits, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually with a total annual cost of more than $3.5 billion. Fortunately, this infection is rapidly responsive to modern antibiotic therapy, so early diagnosis is required to prevent the morbidity. Out of 600 samples screened randomly, 265 (44.2%) were males and 335(55.8%) were females (table 2). Similar to our study, 39.8% male and

60.2% female patients were studied by Dash et al in Odisha, India.[14]Likewise 39.32% male and 56.15% female were enrolled in study by Daniyan S Yand Ojo BA.[15]Of the total study population, majority 179(29.8%) were in the reproductive age group 21-30 years, followed by 89(14.8%) in the age group 41-50 years (table 3). This observation seems to agree with the finding of Obiogbolu GH et al (2009) who found that the incidence of UTI was maximum in the age group of 15 to 40 years.[16] As young women in their sexually active years are one of the most vulnerable group for UTIs, this could be the reason of maximum number of study population in this age group. In present study pyuria was seen in 145 (24.17%) of patients and urine wet mount microscopy was found to be 83.4% sensitive and 82.6% specific with a PPV of 60.5% and NPV of 94%. Similar sensitivity of 85% and specificity of 88% were found by Chenari et al.[17]Studies by Al-Ma'amoory et al,[18] Thakre et al[19] have showed a lower sensitivity of 60% & 72.41% but a higher specificity of 99.2% and 94.47%.The low sensitivity for wet mount examination observed suggests that pyuria is due to bladder colonization by bacteria rather than actual infection. However, this is controversial and probably does not hold true all the time. Besides, hypotonic urine or alkaline urine due to the presence of *Proteus* spp., *Klebsiella* spp. and *Pseudomonas* spp. can cause disintegration of the pus cells. The prevalence of sterile pyuria may be attributed to infections due to organisms like *Chlamydia* spp., which fail to grow in the media used for isolation. Also Thakre et al[19] had screened asymptomatic antenatal females in their study. The patients with asymptomatic bacteriuria often do not excrete an increased number of leucocytes in the urine. So, they might have been under-diagnosed by the urine pus cell count. Also, the absence of pyuria does not exclude infection because patients with neutropenia may have an inadequate white cell response to infection.[20] In our study, out of 600 samples 144 were LET positive; of which 106 were positive by urine culture and 38 were urine culture negative. Out of total 456 samples negative by LET; 417 were negative by urine culture while 39 were positive by urine culture. In comparison with urine culture, sensitivity, specificity, PPV and NPV of LET for detection of bacteriuria and pyuria were 73.1%, 91.6%, 73.6% and 91.4% respectively. Studies by Kacmaz et al[21] and Eyong K et al[22] have shown similar values for sensitivity 70% &71.4% and specificity 92.7% & 92.5% respectively. However studies by Yildirim et al[23], Nayaket al[24], Gayathree et al[25] and Jayalakshmi et al [26] had shown a low sensitivity of 47.7%, 61%, 61.29% and 61.7% respectively. False negative results can occur when large amount of ascorbic acid is present. The high levels of protein (> 300 mg/dL) or glucose (>3 g/dL) and high specific gravity also give false negative LET results as they crenate the white blood cells, leaving them unable to release esterases. Some drugs such as Cephalexin, Tetracycline or high concentrations of oxalic acid may also cause false decrease in leucocyte esterase test results. In our study *E.coli* was reported as the most common aetiological agent of UTI. This is similar to literature where number of studies have reported *E.coli* as predominant organism for UTI by a including both community as well as hospital acquired UTIs, like study by Benwan and collegues for aetiology and susceptibility of CA- UTI and HA-UTI found *E.coli* as the predominant organism.[27]Also, studies by Behzadi,[28]ESCMID group[29]and Gupta UP [30] have shown the similar finding of *E. coli* as most common uropathogen in both CA-UTI and HA-UTI. In contrast to our study, *Staphylococcus aureus* was the commonest pathogen (72%), followed by *Proteus* spp (14%) which were isolated in the study conducted by Akinola et al.[31]Similar results were obtained in study by Omoregie et al, in which *S. aureus* was the predominant isolate.[32]For detection of pyuria both Urine wet mount microscopy and LET have good sensitivity but due to high NPV of 94% , wet mount microscopy can be reasonably used to rule out negative samples. Rapid dipsticks tests for LET are reliable alternative to culture for screening of UTIs. This dipstick test have the advantage of being simple rapid, not requiring any expertise or equipment and costing 1/10th of culture. Therefore, dipstick could be used as an office diagnostic test and in resource limited areas where facility of urine culture is not available. These rapid tests can reasonably screen out bacteriuria and pyuria and reserve routine culture only for those screened positive.

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