

ORIGINAL ARTICLE

DIAGNOSTIC VALIDITY OF LEUKOCYTE ESTERASE DIPSTICK TEST FOR DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOTIC PATIENTS

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Background: Spontaneous bacterial peritonitis (SBP) is defined as an ascitic fluid infection without an evident intra-abdominal surgically treatable source. Spontaneous bacterial peritonitis (SBP) is one of the severe complications in patients with cirrhosis and ascites. Without early antibiotic treatment, this complication is associated with high mortality rate, so early diagnosis and treatment of SBP is therefore necessary for survival. Leukocyte esterase dipstick test can rapidly diagnose the SBP. Objectives were to find out the diagnostic accuracy of leukocyte esterase dipstick test for the diagnosis of spontaneous bacterial peritonitis. **Methods:** This cross-sectional, validation study was conducted from January 2009 to June 2009 at Medical Unit-II, Chandka Medical College Hospital Larkana. All the Patients with cirrhosis and ascites of either gender were included in this study. Paracentesis were performed on admission. The ascitic fluid obtained at bedside was immediately tested with reagent strip Multistix® 10 SG. Ascitic fluid was then analysed for PMN cell count. The result of reagent strip was compared with ascitic fluid PMN cell count for determination of sensitivity, specificity, PPV and NPV of the test, while taking ascitic fluid PMN count $\geq 250/\text{mm}^3$ as standard for diagnosis of SBP. Leukocyte esterase dipstick read positive from +1 to +3 reaction while negative and trace reaction regarded as negative test result. **Results:** Total ninety four patients were enrolled in this study. Ninety-four Ascitic fluid samples were obtained. SBP was diagnosed in 52 (55.3%) patients, 42 (44.7%) patients were negative for SBP by manual cell count. The sensitivity, specificity, PPV, NPV of leukocyte esterase dipstick test to diagnose SBP were 92%, 95%, 96%, 90% respectively. **Conclusion:** The leukocyte esterase dipstick test can be used as rapid test for diagnosis of SBP due to its high diagnostic validity.

Keywords: spontaneous bacterial peritonitis, ascites, cirrhosis, validity, leukocyte esterase.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is defined as an ascitic fluid infection without an evident intra-abdominal surgically-treatable source. Spontaneous bacterial peritonitis (SBP) is one of the severe complication in patients with cirrhosis and ascites. The prevalence of SBP in patients with cirrhosis and ascites ranges from 10% to 30%.^{1,2} This complication is associated with a 30–50% mortality rate³, if not treated and death can occur within few hours. With antibiotics, response can be achieved within 2 days.^{3,4} Early diagnosis and treatment of SBP is necessary for preventing mortality.⁵ Symptoms of SBP are non-specific. These include fever, abdominal pain, nausea and vomiting. Sometimes only hepatic encephalopathy or a precipitating event such as an upper gastrointestinal haemorrhage from ruptured oesophageal varices cause SBP.¹ For these reasons, a diagnostic paracentesis is standard medical practice in any patient with newly diagnosed ascites due to cirrhosis, or in a known patient with ascites who develops symptoms and signs of SBP.¹

An ascitic fluid polymorphonuclear (PMN) count of ≥ 250 cells/ mm^3 is the standard for the diagnosis of SBP¹ irrespective of the ascitic fluid culture, which is variably positive in 40–90% of cases.⁶

The ascitic fluid total leukocyte and PMN count are not always done stat, thereby delaying time to diagnosis. Therefore, a rapid, simple screening test is needed for the prompt diagnosis of SBP. Reagent strip testing for leukocyte esterase activity could be such a test. The test is based on the esterase activity of granulocytes. 3-Hydroxy-5-phenyl-pyrrole esterified with an amino acid is used as the substrate. Hydrolysis of this ester by the esterase releases 3-hydroxy-5-phenyl-pyrrole, which in turn reacts with a suitable diazonium salt, yielding a violet azo dye, the intensity of colour correlates to the leukocyte count.⁷ Use of reagent strip testing for leukocyte esterase has been proposed for the rapid diagnosis of meningitis⁸, urinary tract infections⁹ and peritonitis in patients on peritoneal dialysis¹⁰. Recently, many studies have shown the efficacy of the dipstick test for the diagnosis of SBP.^{11–20} However dipsticks used in these studies are not the same, therefore the diagnostic validity of different dipsticks may vary.

The aim of this study was to determine the diagnostic accuracy, i.e., sensitivity, specificity, positive predictive value, negative predictive value of leukocyte esterase reagent strip test in the bedside diagnosis of spontaneous bacterial peritonitis in cirrhotic patients with ascites, so that rapid diagnosis and treatment can be established.

MATERIAL AND METHODS

The study was conducted in Medical Unit-II, Chandka Medical College Hospital Larkana (CMCH) from January 2009 to June 2009. All the Patients with cirrhosis and ascites of either gender were included in this study (Table-1). Patients with secondary bacterial peritonitis, peritoneal tuberculosis, peritoneal carcinomatosis were excluded Purpose and procedure of paracentesis with its risks and benefits were discussed with patients and finally informed written consent was taken.

The ascitic fluid obtained at bedside was immediately tested in a clean, dry test tube with reagent strip Multistix® 10 SG (Bayer). According to the manufacturer's guidelines for urine testing, strip was immersed in the ascitic fluid, immediately removed, after 120 seconds (the required waiting period), and the colour of reagent square was compared with the colour chart on the bottle. The dipstick was read as either negative or four-tier positive (trace, +1, +2, +3).

The patients were labelled as SBP when dipstick (+1, +2, +3) positive and considered dipstick test positive. The negative and trace result on colorimetric scale was considered negative dipstick test. All ascitic fluid samples were sent to the same laboratory for total and differential cell count.

SPSS-10 was used to analyse data. Relevant descriptive statistics, frequencies and percentages were computed for the presentation of qualitative variables, i.e., sex and viral cause of cirrhosis. Continuous variable, age presented as Mean±SD.

Results of leukocyte esterase reagent strip were compared with ascitic fluid PMN cell Count. The sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) calculated according to their standard formulas by using 2×2 table. Ascitic fluid PMN count $\geq 250/\text{mm}^3$ was taken as standard for the diagnosis of SBP.

RESULTS

Total Ninety-four patients were enrolled in this study and 94 ascitic fluid samples were obtained. Mean age of patients was 44.8±14.9 years, 72 (76.6%) patients were male and 22 (23.4%) were female (Table-1).

Among viral cause of cirrhosis 44 (46.8%) were HBsAg positive, 46 (48.9%) were Anti-HCV positive, 4 (4.3%) patients were both HBsAg and Anti-HCV positive (Table-1).

SBP was diagnosed in 52 (55.3%) patients by manual PMN cell count $\geq 250/\text{mm}^3$. Forty-eight (51%) patients were correctly diagnosed as SBP by leukocyte esterase dipstick test. Among them dipstick reaction was +1 positive in 2 (2.1%) patients, +2 positive in 28 (29.8%), and +3 positive in 18 (19.1%) patients.

Four patients' ascitic fluid PMN cell count was $\geq 250/\text{mm}^3$ but dipstick test was negative. Forty patients' ascitic fluid had negative dipstick test and PMN count was $< 250/\text{mm}^3$, while only two patients' ascitic fluid PMN count was $< 250/\text{mm}^3$ and dipstick test positive. True positive were 48, false positive 2, true negative 40, and false negative were 4.

The sensitivity, specificity, Positive Predictive Value, Negative Predictive Value of leukocyte esterase dipstick test to diagnose SBP were 92%, 95%, 96%, 90% respectively (Table-2).

Table-1: Characteristics of 94 patients with cirrhosis and ascites

| Characteristics | Mean±SD or Number (%) |
|----------------------------------|-----------------------|
| Male | 72 (76.6) |
| Female | 22 (23.4) |
| Age (Mean±SD) | 44.8±14.9 |
| Viral cause of cirrhosis | |
| HBsAg positive | 44 (46.8) |
| Anti HCV Positive | 46 (48.9) |
| Both HBsAg and anti HCV positive | 4 (4.3) |

Table-2: Diagnostic validity of leukocyte esterase reagent strip in diagnosis of SBP

| | Number or % |
|----------------------------------|-------------|
| True positive (n) | 48 |
| True negative (n) | 40 |
| False positive (n) | 2 |
| False Negative (n) | 4 |
| Sensitivity | 92% |
| Specificity | 95% |
| Positive predictive value | 96% |
| Negative predictive value | 90% |

DISCUSSION

Rapid diagnosis of spontaneous bacterial peritonitis is very important in patients with cirrhosis and ascites because of high rate of mortality and morbidity if treatment is delayed.^{2,3} Prevalence of SBP in Pakistan reported from different studies varies, it ranges from 32% to 64%.^{21,22} Currently ascitic fluid PMN count is the standard for diagnosis of SBP.^{1,13-20} Many hospitals in Pakistan have limited laboratory facilities, unable to perform PMN cell count at night, holidays, on weekend and several hours delay in reporting can endanger the patient's life.

Leukocyte esterase enzyme has been shown an important marker for PMN cell activity.²³ The efficacy of leukocyte esterase dipstick test for diagnosis of SBP has been established from many studies done at different centers.¹²⁻²⁰

The present study confirmed the high accuracy of leukocyte esterase dipstick test for rapid diagnosis of spontaneous bacterial peritonitis in cirrhotic patients with ascites.

In this study leukocyte esterase reagent strip correctly detected 48 (51%) positive and 40 (42.6%)

negative cases of SBP from total 94 ascitic fluid samples. Two cases were false positive in which dipstick showed +2 reaction while PMN cell count was 140 and 160/mm³. One possible reason for false positive is +2 reaction correlating with leukocyte esterase reagent strip colorimetric scale PMN cell count that is 125/mm³ which is nearer to manual cell count of the case. False positive results can arise from detection of leukocyte esterase activity in the absence of intact cells. Esterase activity originating other than from leukocyte such as pancreas could produce false positive results.²⁴ There were 4 false negative results in patients with PMN cell count ≥ 250 /mm³. In these cases PMN cell count ranged from 520–1860/mm³, while dipstick reaction was trace in both. In this instance perhaps the PMN were not activated because only activated PMN cell releases the leukocyte esterase,²⁵ this may explain to some extent the observed negative test result. Moreover, antibiotics can produce both false positive and false negative results.²⁶

Results of this study are quite similar to other studies published to date evaluating the use of leukocyte esterase dipstick test in the diagnosis of SBP (Table-3). Butani *et al*²⁰ used leukocyte esterase reagent strip to diagnose SBP in 136 specimens by using grade 2 as a cut-off scale, and found the sensitivity, specificity, PPV and NPV of the leukocyte esterase reagent strip as 83%, 99%, 91%, and 98% respectively. In study done by Vanbiervliet *et al*, nine of 72 patients included were diagnosed with SBP, another leukocyte esterase reagent strip was positive in all cases with 100% sensitivity and specificity.¹⁴ Sapey *et al* found sensitivity, specificity, PPV, NPV of leukocyte esterase reagent strip as 64.7%, 99.6%, 91.7%, and 97.4% respectively.¹⁹ Kim *et al* revealed 50% sensitivity, 100% specificity, 100% PPV and 87% NPV of the leukocyte esterase reagent strip in his study.¹⁸ Thevenot *et al* found 89% sensitivity, 100% specificity, 100% PPV and 99% NPV.¹⁷

Different dipsticks have their own diagnostic validity. To date, there has been no dipstick that was

specifically designed to use in ascitic fluid. Moreover, many dipsticks do not have a precise colorimetric scale for 250 PMN cells/mm³. Castellote *et al*¹⁵ studied the use of Aution sticks (A. Menarini Diagnostics, Firenze, Italy) for diagnosis of SBP in cirrhotic patients with ascites who underwent abdominal paracentesis at a university based hospital, and found that the sensitivity, specificity and PPV are 89%, 99% and 98%, respectively. The benefit of the Aution stick is the precise colorimetric scale that correlates with ≥ 250 PMN cells/mm³.

Sarwar *et al* used Combur 10 urine strip for diagnosis of SBP and found 97.7% sensitivity and 89.4% specificity with positive predictive value of 90%, negative predictive value of 97.7%.²⁷

There is great potential in using leukocyte esterase reagent strips for diagnosis of SBP. The reagent strips are easy to use, do not require expertise, are rapid, can be performed bedside, and have high sensitivity and specificity for diagnosing of SBP, thereby allowing antibiotic therapy to start immediately. Another noteworthy point is the low cost of the strip. This test could be performed by the physician, house officer, nurse, or any other qualified technician collecting the ascites fluid. The result of the test could initiate appropriate management while standard ascitic fluid analyses are pending. Thus the use of this diagnostic modality could save lives by prompting early therapy. This method is also useful for determining the effectiveness of the antibiotic therapy.

High NPV of dipstick test could make it useful bedside screening tool, especially in ambulatory setting like OPD and emergency room. Patients with negative testing could be quickly discharged. Conversely high PPV of strip warrants immediate start of antibiotics in case of positive result. Considering the mortality from SBP, this test will help to improve the management of SBP.

Table-3: Validity scores reported in previous studies

| Authors | Vanbiervliet <i>et al</i> | Kim <i>et al</i> | Butani <i>et al</i> | Sapey <i>et al</i> | Thevenot <i>et al</i> |
|-----------------|-----------------------------|-----------------------------|------------------------------|------------------------------|-----------------------------|
| Dipsticks | Multistix [®] 8 SG | Mutistix [®] 10 SG | Multistix [®] 10 SG | Multistix [®] 10 SG | Multistix [®] 8 SG |
| Sensitivity (%) | 100 | 50 | 83 | 65 | 89 |
| Specificity (%) | 100 | 100 | 99 | 100 | 100 |
| PPV (%) | - | 100 | 91 | 92 | 100 |
| NPV (%) | - | 87 | 98 | 97 | 99 |

CONCLUSION

The leukocyte esterase dipstick test has high sensitivity, specificity, positive predictive value and negative predictive value for diagnosis of spontaneous bacterial peritonitis (SBP) in cirrhotic patients with ascites. It can be used at bedside, easy to perform, is rapid and inexpensive. A positive test result is indication for empirical antibiotic therapy and a negative test result excludes SBP.

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