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Diagnostic evaluation of leukocyte esterase reagent strip in bedside rapid diagnosis of spontaneous bacterial peritonitis in cirrhotic patients

Kavitha B[1], Rajalakshmi E[2], Vinita Agarwal[3], Balasubramanian R[4]

[1]Professor, [2,4]Associate Professor, Department of General Medicine, [3]Associate Professor, Department of Pathology, Indira Gandhi Medical College and Research Institute, Puducherry, India

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ABSTRACT

Background: Spontaneous bacterial peritonitis (SBP) is the most common and serious infection of liver cirrhosis patients with ascites. If not intervened with early antibiotic treatment leads to high morbidity and mortality. Thus, early diagnosis and treatment of SBP are needed for survival. Leukocyte esterase reagent can aid in early diagnosis. **Aims and Objectives:** This study aimed to assess the diagnostic accuracy of the leukocyte esterase dipstick test for the diagnosis of SBP in resource poor settings. **Materials and Methods:** This cross-sectional study was conducted during August 2018–January 2019 on patients with cirrhotic liver disease and ascites in a tertiary care medical college hospital, Puducherry. All patients underwent abdominal paracentesis, and ascitic fluid was subjected to cell counts, biochemistry tests, culture, and leukocyte esterase test (Multistrix SG8- Seimens) at 120 s and graded with five levels. Grade 3 was positive (125 polymorphonuclear leukocytes [PMNL]/mL) and grade 4 (<500/mL), which exceeds the threshold for SBP (<250 PMNL/mL). Sensitivity, specificity, positive, and negative predictive value (PPV and NPV) was calculated. **Results:** A total of 50 ascitic fluids were analyzed. Nine out of 50 (18%), 16 (32%), and 4 (8%) were positive for cell count method, Leucocyte esterase reagent (LER) test and culture, respectively. The sensitivity, specificity, PPV, NPV, and diagnostic accuracy for LER test were 6.25%, 91.18%, 25%, 67.39%, and 64%, respectively. **Conclusion:** The LER strip test is useful for SBP diagnosis in an emergency and resource poor setting. It provides good diagnostic accuracy and high NPV and it is cost effective and better bedside tool for SBP diagnosis.

Key words: Ascites; Peritonitis; Liver cirrhosis; Neutrophils; Esterases; Leukocyte esterase

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INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a very common and most serious complication of patients with liver cirrhosis having ascites. SBP is characterized by impulsive infection of the ascitic fluid without an intra-abdominal source of main foci. SBP can occur in 10–30% of cirrhotic patients.[1] In most situations, the in-hospital mortality rate stays between 30 and 50%.[2] The gold standard test for diagnosing SBP is by demonstrating a polymorphonuclear leucocyte (PMNL) count of >250/μL in the ascetic fluid in liver cirrhosis patients.[1] This may be time consuming and requires special equipment and lab technicians, who may not be available round the clock. Ascitic fluid culture also needs time and the rate of positivity is quite less, 60% of the SBP cases the culture is negative.[2,3] The delay in diagnosing and initiating appropriate antibiotics may push the patient to a higher risk of sepsis and mortality. Hence the need for a rapid, bedside, efficient, and simple test to diagnose SBP instantaneously at the bedside will be helpful to treat SBP early.

LER strips were developed originally to detect PMNL in urine which proved to be accurate. Similarly, it would

Address for Correspondence:
Dr. Balasubramanian R, Associate Professor, Department of General Medicine, Indira Gandhi Medical College and Research Institute, Puducherry - 605 009, India. **Mobile:** +91-9443436603. **E-mail:** drbala71@yahoo.co.in

be beneficial for detecting PMNL in bodily fluids other than blood as pleural fluid, cerebrospinal fluid, and ascitic fluid.[4,5] Studies conducted in developed countries suggested that the LER strip test significantly reduced the diagnosis time from hours to minutes.[6] In this assay, PMNL esterase activity in the fluid interacts with a substrate ester that releases 3-hydroxy-5-phenylpyrrole; this causes the azo dye on the reagent strip to shift color, the intensity of color of the strip used correlates to the leukocyte count.[5] Many studies reported sensitivity and a specificity of 100% for the strip test (Multistix 8SG) in the analysis of SBP.[5-7] However, in 2007, a multicentric (70 centers) study which was prospective in nature conducted with a total of 2123 paracenteses performed in 1041 patients was published stating very high specificity for LER strip test in the diagnosis of SBP but exposed a poor sensitivity of 45% only.[8] Hence, there is no clear consensus on the role of the LER strip test. Since the results are conflicting, this article propose to carry out this study to assess the diagnostic accuracy of the LES strip test in a resource poor setting.

Aims and objectives

To compare the diagnostic efficacy of Leukocyte Esterase Reagent (LER) strip test with ascitic fluid cell count method among cirrhotic patients with suspected SBP.

MATERIALS AND METHODS

This prospective observational study was conducted in the department of medicine in a tertiary care medical college hospital in Puducherry from August 2018 to January 2019. This study included adult (above 18 years old) patients with liver cirrhosis and ascites before the initiation of antibiotics. After obtaining permission from the Institutional Ethics Committee 159/IEC/IGMC and RI/F-7/2018] and written informed consent from the participants, this article included all cirrhosis patients diagnosed using conventional clinical, biochemical, and ultrasonographic criteria during the study period. Exclusions were made for cases of secondary peritonitis, peritoneal TB, peritoneal carcinomatosis, pancreatic ascites, and those on prophylactic antibiotics for SBP or prior history of SBP.

Paracenteses

As prescribed by standard medical protocol, paracenteses were conducted routinely upon admission and were repeated when necessary. As it is standard practice, every sample of ascitic fluid was subjected to a manual cell count, differential cell count (using a light microscope ascitic and percentage of PMNL were determined), and biochemistry (total protein, albumin, and sugars). All patients had cultures of ascitic fluid conducted at the bedside using blood culture vials containing both aerobic and anaerobic media and 10 mL of inoculum. Age, gender, ethnicity, origin of cirrhosis, severity of cirrhosis, complications of cirrhosis other than ascites or SBP, history of SBP prophylactics or empiric treatment, and all ascites fluid analysis results were collected from the study participants.

Leukocyte esterase reagent strips

The sample of ascitic fluid was collected in a dry, sterile test tube. The strip was promptly removed from the ascitic fluid when all reagent regions were immersed in it. The color of the reagent square corresponding to leukocytes was compared to the color chart on the bottle after the required waiting period. The MultistixSG 8 (SEIMENS) is evaluated at 120 s and is graded with five levels starting from 0,+1 to +4.[9,10] The strip was deemed positive at Grade 3 (125 leukocytes/mL) since Grade 4 (<500/mL) exceeds the threshold for SBP (<250/mL), because the PMNL count of the majority of individuals with SBP caused by Gram-positive cocci is <250/mL.[11] Two investigators tested the strips separately, neither of whom knew the results of the ascitic fluid cytological testing at the time of reading. In the event of disagreement between the two readers, the greater value was recorded. Antibiotic treatment was commenced empirically in all cirrhotic patients with an ascitic fluid PMN cell count of more than 250 cells per mL.

In all patients, the results of the leukocyte esterase reagent strip test were compared to PMNL counts, culture of ascitic fluid, biochemistry analysis, and clinical data. The validity of the test by sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of each reagent strip for the diagnosis of SBP was evaluated. The SBP was defined as a PMN count of more than 250 cells per mL. Probability ratios were computed as follows: likelihood of a positive test result likelihood ratio for a positive result (LR+) = sensitivity/(1-specificity); likelihood ratio for a negative result (LR–) = (1-sensitivity)/specificity. $P<0.05$ was considered significant statistically. The data were analyzed using SPSS software, V.22.[12]

RESULTS

Finally, a total of 50 individuals were included in the analysis. The mean age was 51.58±11.69 years. The majority of 44 (88%) participants were males and 6 (12%) were females. As per etiology, the majority had alcoholic liver cirrhosis 27 (53.4%) followed by hepatitis 14 (28%), and others. The majority of (93.75%) participants had abdominal distension, 52.08% had pedal edema, and 31.25% had abdominal pain. Out of 50 participants, 46 (80%) participants did not have hepatic encephalopathy (HE) while 6 (12%) had HE grade 1. Out of 50 participants, the majority of 28 (7.14%) participants had moderate

Table 1: Summary of laboratory investigation of the study participants

Parameter	Median (IQR)	Minimum	Maximum
Total cell count (n=50)	8150 (5,600–11,000)	1100.0	19000.0
Neutrophils (n=50)	79.5 (70–83)	62.0	92.0
Lymphocytes (n=50)	17 (12.75–26)	1.0	40.0
Platelets (n=50)	79,500 (58,000–105,750)	8600.0	656000.0
Liver function test			
Total bilirubin (n=50)	3.25 (1.775–5.4)	0.5	21.2
Indirect bilirubin (n=50)	1.4 (0.8–3.05)	0.3	11.2
Direct bilirubin (n=50)	1.6 (0.7–3.3)	0.2	10.5
Total protein (n=50)	6.1 (5.6–6.7)	4.0	7.9
Albumin (n=50)	2.4 (2.2–2.725)	1.5	3.7
Globulin (n=50)	3.6 (3.175–4.15)	2.2	5.2
AST (n=50)	74.5 (46.75–115)	29.0	710.0
ALT (n=49)	35 (24.5–56.5)	14.0	360.0
ALP (n=50)	223.5 (172.5–325.5)	70.0	1496.0
INR (n=47)	1.8 (1.5–2.3)	1.0	4.0
PMNs	7 (0–40.5)	0.00	22950.00
Neutrophils	5.5 (0–30.5)	0.00	90.00
Leucocytes	70 (50–90)	1.00	100.00

AST: Aspartate aminotransferase, ALT: Alanine transaminase test, ALP: Alkaline phosphatase, INR: International normalized ratio, PMNs: Polymorphonuclear neutrophils, IQR: Interquartile range

ascites and 17 (34.69%) had mild ascites. As per HRS, a history of SBP was reported in 18% (9 out of 50) and ultrasound abdomen findings were cirrhosis of the liver 90.63% (29 out of 32). Ascitic fluid was straw colored in 95.83% and Child Pugh score was B in 9 out of 50 (18%).

All laboratory parameters are tabulated in Table 1 with median and interquartile ranges for each parameter measured in the study individual.

Out of 50 participants, 9 (18%) were positive as per the ascitic fluid cell count method, 16 (32%) were positive in LER grade, and 4 (8%) were positive according to the culture report (Table 2). In this article, positive and negative LER tests were analyzed individually based on PMN count and culture. Leukocyte esterase test findings in terms of the distribution and frequency of PMNL cell counts. The sensitivity of 6.25% (95% CI 0.16–30.23%) was noted in the ascitic fluid cell count method and LER grade for each respectively in predicting culture report. However, the diagnostic accuracy was 70% based on the ascitic fluid cell count method and it was 64% in LER grade since the specificity of LER grade was 91.19% is more compared to the ascitic fluid cell count method with 88.24% (Tables 3 and 4).

Table 2: Summary of three different methods (ascitic fluid cell count method, leucocyte esterase reagent strip grading method, and culture method)

Parameter	Frequency (%)
Ascitic fluid cell count method	
Positive PMN (>250 mL)	9 (18)
Negative	41 (82)
LER strip grading method	
Positive (> grade 3)	16 (32)
Negative	34 (68)
Culture method	
Positive	4 (8)
Negative	46 (92)

PMN: Polymorphonuclear, LER: Leucocyte esterase reagent

Table 3: Comparison of culture report with ascitic fluid cell count method and leucocyte esterase reagent grade

Parameter	LER strip method	
	Positive (n=16)	Negative (n=34)
Ascitic fluid cell count method, n (%)		
Positive	5 (31.25)	4 (11.76)
Negative	11 (68.75)	30 (88.24)
Culture report, n (%)		
Positive	1 (6.25)	3 (8.82)
Negative	15 (93.75)	31 (91.18)

LER: Leucocyte esterase reagent

DISCUSSION

SBP is one of the most important causes for both morbidity and mortality among cirrhotic patients with ascites.[13] Therefore, early intervention of prompt diagnosis and treatment of these patients is crucial for enhancing their clinical results. Methods typically utilized tests in the diagnosis of SBP take longer time which takes from few hours to many days.[14] In addition to this, ascitic fluid cell count is measured by many techniques such as hematological, optical microscopy, and manual counting. Furthermore, the ascitic PMN count is measured by hematological techniques, optical microscopy, and manual counting. Even though polymorphonuclear cell count of ascitic fluid is the standard method in the diagnosis

Table 4: Predictive validity comparison of ascitic fluid cell count method and leucocyte esterase reagent Strip test method in predicting culture report

Parameter	Ascitic fluid cell count method			LER grade		
	Value	95% CI		Value	95% CI	
		Lower	Upper		Lower	Upper
Sensitivity, n (%)	31.25	11.02	58.66	6.25	0.16	30.23
Specificity, n (%)	88.24	72.55	96.70	91.18	76.32	98.14
False positive rate, n (%)	11.76	3.30	27.45	8.82	1.86	23.68
False negative rate, n (%)	68.75	41.34	88.98	93.75	69.77	99.84
Positive predictive value, n (%)	55.56	21.20	86.30	25.00	0.63	80.59
Negative predictive value, n (%)	73.17	57.06	85.78	67.39	51.98	80.47
Diagnostic accuracy, n (%)	70.00	55.39	82.14	64.00	49.19	77.08
Positive likelihood ratio	2.66	0.17	3.779	0.71	0	0.835
Negative likelihood ratio	0.78	0.26	1.108	1.03	0.44	1.212

LER: Leucocyte esterase reagent, CI: Confidence interval

of SBP, it is proven to be a tedious and heavily time-consuming procedure.[1] Protracted SBP diagnosis can result in a worsening of the disease's progression and clinical repercussions.[2] Due to these issues, significant attempts were made over the past few years to create an alternate trial for a quicker diagnosis of SBP. Leukocyte esterase enzyme has been demonstrated to be a significant indicator of polymorphonuclear cell activity.[3,4] The effectiveness and efficiency of the LER test for the diagnosis of SBP have been recognized in prior research.[4-6]

This article confirmed the diagnostic worth of LER strip method, out of 50 samples, 16 (38%) turned to be positive for SBP, however, sensitivity was only 6.25% and specificity was 91.18%. However, this test had NPV (67.39%) but diagnostic accuracy is low with 64%. It can imply a tentative, but early diagnosis of SBP. It helps to start antibiotic medication for SBP, whereas polymorphonuclear cell counts and confirmatory culture and sensitivity reports were awaited.

Despite the fact that numerous researchers have analyzed the diagnostic accuracy of the LER strip test in SBP studies, ironically, the outcomes of these studies differ. In a study conducted by Farahmand et al., nine cirrhotic liver patients were examined, and the study's sensitivity, specificity, positive predicate value, and negative predicate value were all calculated to be 100%.[15] Other research on adults has reported varying levels of reporting sensitivity and specificity, for instance, out of 63 patients, 15 participants were diagnosed to have SBP in research conducted by Torun et al.,[16] the results of sensitivity, specificity, PPV, and NPV of the LER strips were 93%, 100%, 100%, and 98%, respectively. In another study conducted by Vanbiervliet et al., included 72 patients with cirrhosis, out of them nine individuals were diagnosed with SBP, and the LER test was found to have 100% of diagnostic accuracy with cent percent sensitivity and specificity.[17] Butani et al., utilized LER strip test tool to evaluate SBP in 136 individuals, study concluded the sensitivity, specificity, PPV, and NPV values as 83%, 99%, 91%, and 98%, respectively.[10] In 94 cirrhotic patients, Khatwani et al., observed that the sensitivity, specificity, PPV, and NPV of the LER strip test to detect SBP were 92%, 95%, 96%, and 90%, respectively.[18] Despite the fact that numerous studies have been done on the application of the LER test in SBP, the outcomes of these investigations vary. Dissimilarities in the results of the LER strip test can be partially explained by the use of distinct kinds of reagent strips and the application of distinct limitation range values.

Limitations of the study

Even though this study stands out to be the only source of data from southern India, it is a single-center study with a small sample size. In addition, inclusion criteria were stringent as it included individuals who had taken antibiotics before being admitted to the hospital were not included. Colorimetric scale interpretation can be subjective in the author's opinion/experience; a single examiner was used to eliminate inter-observer variability. Consequently, intra-observer variability and inter-observer variability are both possible in this study.

CONCLUSION

The present study demonstrated that the usage of LER strip test for diagnosing SBP provides better diagnostic accuracy and good NPV for SBP diagnosis, and also the advantages of time minimizing, cost reduction, availability at odd hours, and no requirement for specialist training. The rapid nature of this test may facilitate the initiation of antibiotic treatment in individuals with SBP.

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ETHICS STATEMENT

Ethical approval was obtained from the institutional review board [Ref: 159/IEC/IGMC&RI/F-7/2018] of the center concerned. Informed written consent was obtained before the study started and confidentiality was maintained throughout.

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Authors' Contributions:
BR - Has conceptualized the study and played a primary role in compiling, analyzing, and interpretation of the data. All the drafts were prepared, reviewed, and final draft was approved by **KB, R, VA, BR -** Have contributed in fine tuning of the proposal, contributed in data collection and entry. Reviewed the results and contributed to preparation and review of drafts. All the authors have read and approved final version of the manuscript. All the authors take complete responsibility for the content of the manuscript.

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ORCID ID:
Dr. Kavitha B - https://orcid.org/0000-0002-6710-4854
Dr. Rajalakshmi - https://orcid.org/0009-0005-7091-497X
Dr. Balasubramanian R - https://orcid.org/0009-0009-3531-8311

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