EVALUATION OF LEUKOCYTE ESTERASE REAGENT STRIP TEST FOR RAPID BEDSIDE DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS

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ABSTRACT

Spontaneous bacterial peritonitis (SBP) is a common complication of cirrhosis with high morbidity and mortality. Early diagnosis and treatment improve survival. Diagnosis is made by ascitic fluid Polymorphoneuclear leukocytes (PMNL) count of >250/mm3 which takes hours and may not be available in rural settings. Leukocyte esterase reagent strips (LERS) test have shown high sensitivity, specificity, and negative predictive value in the diagnosis of SBP. This study was conducted to find the utility of the LERS test for the diagnosis of SBP. This was a prospective hospital-based study conducted at the National Academy of Medical Sciences, Bir Hospital, Nepal. LERS test was performed on ascitic fluid from 140 cirrhotic patients. Colorimetric grading was compared with PMNL count for diagnosing SBP. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for diagnosing SBP calculated for grade 3 and grade 4 as cut-offs. Among 140 patients, SBP was diagnosed in 27. Grade 3 as cut-off, sensitivity, specificity, PPV, and NPV for the LERS test were 96.3, 90.2, 70.3, and 99 percent respectively. For grade 4 as cut-off, sensitivity, specificity, PPV, and NPV were 81.5, 99.1, 95.6, and 95.7 percent respectively. Accuracy were 91.4 and 95.2 percent for grade 3 and 4 as cut-off respectively. LERS test has shown high sensitivity, specificity, and negative predictive value for the diagnosis of SBP in cirrhotic ascitis. Being simple, rapid, and cost-effective, it can be useful at bedside to start early antibiotic therapy before availability of the PMNL count report.

KEYWORDS

Cirrhosis, leukocyte esterase reagent strips, PMNL count, spontaneous bacterial peritonitis

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INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is defined as bacterial infection of ascites fluid without any intra-abdominal source of infection. SBP may be asymptomatic or may present with various symptoms of peritonitis, systemic inflammation, or worsening liver functions. Diagnosis of SBP is made with the ascitic fluid polymorphonuclear leukocyte (PMNL) count greater than 250/mm3.[1] The prevalence of SBP is low (3.5% or lower) in asymptomatic outpatients, but increases to 8% to 36% in admitted patients with half of the episodes acquired during hospitalization.[2] With early diagnosis and treatment, mortality from SBP has decreased from 90% to 20% in recent times.[1] Each hour of delay in diagnosis is associated with a 3.3% increase in in-hospital mortality.[3] Facilities to perform rapid total and differential leukocyte count may not be readily available, especially in rural settings. Beyond office hours, cell count may not be available within few hours and the test is prone to human errors along with intraoperator and interoperator variability.[2,4,5]

Initially leukocuye esterase reagent strips (LERS) was developed to test for PMNL in urine.[6] Usefulness of LERS has also been shown in detecting PMNL in other body fluids such as pleural fluid, and cerebrospinal fluid.[7,8] In this test 3-hydroxy-5-phenyl-pyrrole esterified with an amino acid is used as substrate and hydrolysis of this ester by the esterase of PMNL results in the release of 3-hydroxy-5-phenyl-pyrrole, which in turn reacts with a suitable azo dye in reagent strip to cause color change and the intensity of color change is read against standard chart.[9] Though it is cost-effective, easily available, less time-consuming, and can be performed at the bedside in comparison to the cell counting method; its usefulness in the diagnosis of SBP is variable with sensitivity ranging from 45 to 100% and specificity of 42 to 100%.[10,11] The aim of this study is to determine the accuracy of the LERS test for the diagnosis of SBP in patients with cirrhotic ascites.

MATERIALS AND METHODS

This was a hospital-based observational analytical study conducted at the National Academy of Medical Sciences, Bir Hospital, Kathmandu from September 2021 to July 2022. Ethical approval was obtained from the Institutional Review Board of NAMS (Ref. No. 1193/2078/79). Informed consent was taken from all the participants. The diagnosis of cirrhosis with ascites was made based on clinical, biochemical, and ultrasonography findings. Convenience sampling was used to collect data.

Sample size was calculated by using the following formula:

$$n = \frac{Z^2_{1-\alpha/2} \times S_N \times (1-S_N)}{L^2 \times Prevalence}$$

Where n = required sample size,

S_N = anticipated sensitivity,

L = maximum clinically acceptable width of 95% CI,

α = size of the critical region (1 − α is the confidence level),

$Z_{1-\alpha/2}$ = standard normal variate corresponding to the specified size of the critical region (α), and

Standard normal variate at 5% type 1 error (P<0.05) is 1.96. Thus taking standard normal variate of 1.96, maximum clinically acceptable width of 10%, and expected proportion of SBP of 13.3 % as from a study done in Nepal[12], and sensitivity of the test 95%[5] the calculated sample size was 140.

Abdominal paracentesis was performed with strict aseptic precaution in all cirrhotic patients with new onset of ascites, and hospitalized patient with either of worsening ascites or any complications of cirrhosis with or without clinical signs of SBP. Immediately after the paracentesis, ascitic fluid (AF) was tested using LERS (YERCON URS-10A Reagent strips, Yercon Diagnostic Co. Ltd) by immersing it in 5 mL of fluid placed in a dry and clean container. After 2 minutes, the LERS color change was compared with the colorimetric scale depicted on the bottle, and grading was done from grade 0 to grade 4 as suggested by the manufacturer as follows: grade 0:0 PMNL/μL, grade 1: 15 PMNL/μL, grade 2:70 PMNL/μL, grade 3:125 PMNL/μL and grade 4:500 PMNL/μL. Two different cut-offs, grade 3 and grade 4 were tested. 10 ml fluid was sent for PMNs count and biochemical analysis (Total protein, albumin, glucose, LDH). Ten ml fluid was inoculated for culture. The diagnosis of SBP was based on a PMNL count >250/mm^3 in ascitic fluid, irrespective of a positive culture and clinical signs of SBP and in absence of intra-abdominal sources of infection, inflammation or tuberculosis. Appropriate treatment was started as per standard protocol.

Demographics were analyzed in frequencies, percentages, or mean (±standard deviation). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of Leukocyte reagent strip at different

cut off (grade 3 and grade 4) were calculated in comparison to diagnosis of SBP made by PMNL count. Receiver operating characteristic (ROC) curve was elaborated for two cut-off. Data were analyzed using SPSS version 23.0.

RESULTS

Table 1: Characteristics of the study population

Variable	Mean±SD or n (%)
Age (years)	50±10
Sex (M/F)	107/33 (76.43%/23.57%)
Child-Pugh class	
A	3 (2)
B	60 (43)
C	77 (55)
Etiology	
Alcohol	114 (81.4)
Hepatitis B	6 (4.2)
Hepatitis C	4 (2.8)
Non-alcoholic fatty liver disease	7 (5)
Others*	3 (2.14)
Unknown	6 (4.2)

*1-Hemochromatosis, 1-Budd-Chiari syndrome, 1-Cardiac cirrhosis

Table 2: Prevalence of SBP (PMNL>250/mm^3)

Diagnosis	n (%)
Ascites without SBP	113(80.7)
Ascites with SBP	27(19.3)
Culture positive	11
Culture negative	16

Total 140 patients were studied. Mean age was 50(±10) years with male predominance, majority were Child-Pugh class B or C. Alcohol was the commonest etiology. The baseline characteristics were as shown in Table 1.

Median CTP score was 10. 27 (19.3%) patients were diagnosed as SBP based on PMNL count (Table 2). Ascitic fluid culture was positive in 11 (40.7%) of 27 patients, with *Escherichia coli* in 6 cases, *Klebsiella pneumoniae* in 3, *Streptococcus pneumoniae* in 1 and *Acinetobacter baumannii* in 1.

Ascitic fluid protein was lower in those with SBP with mean of 1.02gm/dl in comparison to 1.52gm/dl in patients without SBP. On LERS testing 34 patients and 48 patients showed grade 0 and grade 1, respectively and none had SBP. Out of 21 patients with grade 2 LERS result 1 had SBP. Four out of 14 patients with grade 3 and 22 out of 23 patients with grade 4 LERS result had SBP respectively (Table 3).

Table 3: Result of LERS test compared to SBP diagnosis based on PMNL count

Diagnosis(based on PMNL count)	LERS test Result(In grade)					Total
	No			Yes		
	0	1	2	3	4	
SBP	0	0	1	4	22	27
No SBP	34	48	20	10	1	113

Table 4: Performance characteristics of LERS, Using two different cut-offs for diagnosis of SBP

Variable	Grade 3 as a cut-off	Grade 4 as a cut-off
Sensitivity	96.3	81.5
Specificity	90.2	99.1
Positive predictive value	70.3	95.6
Negative predictive value	99	95.7
Accuracy	91.4	95.7

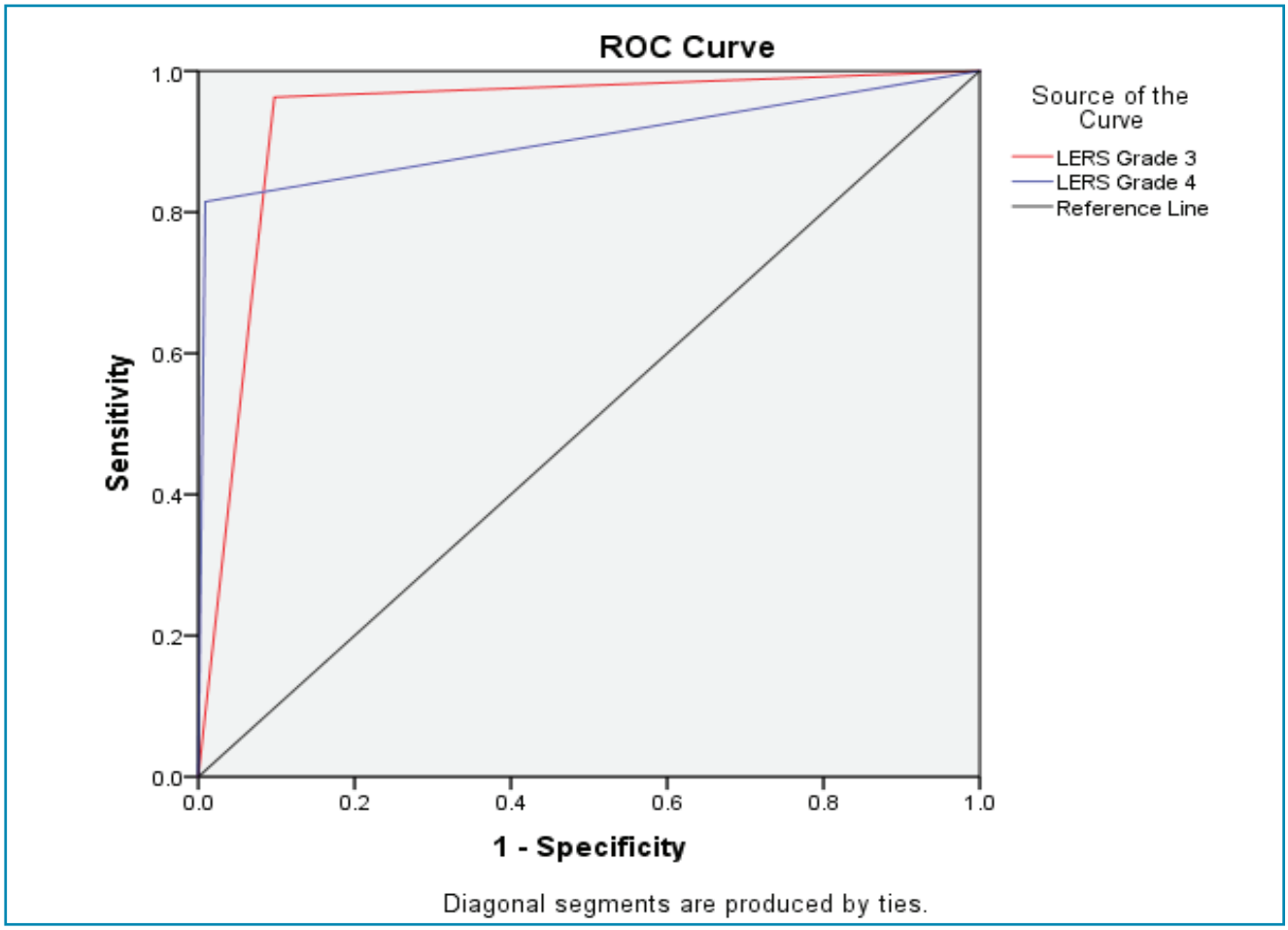

Fig. 1: ROC curve of LERS taking grade 3 and 4 as cut-off for SBP diagnosis

The performance of LERS test at two cut-off were compared with PMNL count. At cut-off of grade 3 for diagnosis of SBP sensitivity, specificity, PPV, and NPV were 96.3, 90.2, 70.3 and 99 respectively. At cut-off of grade 4 sensitivity decreased to 81.5, whereas specificity and PPV improved to 99.1 and 95.6 respectively with NPV of 95.7. Overall accuracy at grade 3 and grade 4 as cut-off were 91.4 and 95.7 respectively (Table 4).

ROC curve had area under the curve of 0.933 and 0.903 at the cut-off of grade 3 and 4 respectively (Fig. 1).

DISCUSSION

Since delay in diagnosis of SBP and institution of antibiotics increases the mortality, test for rapid diagnosis is very important. At present ascitic fluid PMNL count of ≥250/mm^3 is the standard tool for SBP diagnosis and initiation of antibiotics without waiting for culture report is a common practice.[1] However, PMNL count alone also takes hours and may not be available in remote areas and small outpatient settings.[13]

In our study, usefulness of LERS test on ascitic fluid for diagnosis of SBP has been demonstrated. Of the two cut-offs, cut-off of grade 3 has shown higher sensitivity (96.3%) but lower specificity (90.2%) compared to cut-off of grade 4 with lower sensitivity (81.5%) and higher specificity of (99.1%). Similarly grade 3 cut-off had lower PPV (70.3%) and higher NPV (99%) in comparison to grade 4 cut-off (PPV and NPV of 95.6% and 95.7% respectively) but lower accuracy (91.4% vs 95.2%). Performance of both the cut-offs were reasonably good for diagnosis of SBP.

In a study by Khairnar *et al*[14] done in 64 patients, 17 with SBP, using multistix 10SG (Siemens India), had shown the sensitivity, specificity, PPV, NPV and accuracy of 100, 94, 57, 100 and 94.5 percent with cut-off grade 2 (:125 PMNL/mm^3) and 76.4, 100, 100, 92.1 and 93.7 percent respectively with grade 3 (:500PMNL/mm^3) as cut-off. The finding in this study showed high sensitivity and NPV with low specificity for grade 2 as cut-off and specificity and PPV improved at cost of sensitivity for grade 3 as cut-off and these finding are similar to our study. Castellote *et al*[15] used Aution sticks in

228 ascitic fluid with 52 having SBP, shown high sensitivity of 96 and 89%, specificity of 89 and 99% and NPV of 99 and 97% for grade 2 (:75PMNL/mm^3) and grade 3 (:250PMNL/mm^3) respectively.

In another study by Balagopal *et al*[13] including 175 patients (75 with SBP) using Magistik-10(Peerless Biotech Lab) had higher sensitivity of 97 and 92% for grade 2 (>125PMNL/mm3) and similar specificity of 89 and 100% for grade 3 (>500PMNL/mm^3) respectively. In contrast to our result Jha *et al*[16] had shown lower sensitivity of 77.7 and 66.6% for grade 2 and grade 3 cut-off, respectively.

The differences in performance of LERS strips in different studies has also been observed in a systemic review by Koulaouzidis *et al*[11] which included 17 studies comprising of total of 2625 patients and a total of 4930 paracenteses with total of 535 episodes of SBP. Leucocyte esterase reagent strips compared with the manual polymorphonuclear count ('gold standard') was found to have sensitivity ranging from 45 to 100%, specificity ranging from 81 to 100%, positive predictive value ranging from 42 to 100% and negative predictive value ranging from 87 to 100%. They concluded that despite differences in sensitivity and PPV between different studies, there is consistently excellent NPV of reagent strips in SBP diagnosis.These differences in result may be due to use of different grades as cut-offs; as well as the use of LER strips made by different manufacturers. Sapey *et al*[17] tested the performance of two brands of strips (Nephur-test and multistixSG10) in a total of 245 samples, with SBP in 17. They documented sensitivity of 88.2% for Nephur-test and 64.7% for multistixSG 10.

Considering the high mortality rate of SBP, a lower cut-off is preferable as it enhances the sensitivity and NPV in expense of slight decrease in specificity, so that cases of SBP are not missed. In our study both the cut-off grades performed reasonably well though the cut-off of grade 3 had high sensitivity and NPV with slight loss of specificity with similar accuracy in comparison to grade 4 as a cut-off. By virtue of its low cost, rapidity in the yield of results, ease of use, wide availability and reasonable accuracy, LERS test can be an useful alternative in screening and diagnosing SBP.

There are few limitations in our study. First, the LER strip used in this study does not have cut-off that corresponds to cell count of 250 PMNL/mm^3 which defines SBP. Reading of colorimetric scale was performed by single observer so possibility of inter-observer variation cannot be denied. It was a single center study with relatively smaller sample size with use of one manufacturer strips.

In conclusion, this study shows that LER strip test has high sensitivity, specificity and NPV in diagnosis of SBP in cirrhotic patients with ascites. This test can be useful at bedside to start early antibiotic therapy before the availability of PMNL count report. Before making recommendation to use LERS test as an alternative to gold standard of PMNL count in ascitic fluid, it is suggested that further studies with larger sample sizes with use of LER strips from different manufacturers (with a cut-off grade corresponding to 250 PMNL/mm3) are performed to substantiate the promising result as we observed.

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