Efficiency Assessment of Leucocyte Esterase Reagent strips in rapid bedside diagnosis of Spontaneous Bacterial Peritonitis - a comparison study with the gold standard Absolute Neutrophil Counts in ascitic fluid

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ABSTRACT **BACKGROUND** *Spontaneous Bacterial Peritonitis(SBP) is a well known complication of cirrhotic ascites. An early diagnosis is essential to reduce the morbidity and mortality. Identifying a rapid and affordable bedside tool which can help in diagnosis of SBP was the objective of our study.*

METHODS *A prospective study of 200 patients admitted with ascites in a tertiary care teaching hospital during a two year period was done. The incidence of SBP in those patients and its diagnosis based on ascitic fluid neutrophil counts and cultures were recorded. We compared the LER strips colour change with the absolute neutrophil counts in the ascitic fluid which is the gold standard.*

RESULTS *180 out of 200 patients with ascites were cirrhotic in nature. 89% of the study group were males. Ethanol induced chronic liver disease was the commonest etiology. SBP was seen in 17.8% cases and fever was the commonest presentation. 5 out of 32 cases of SBP were culture positive. The sensitivity and specificity of LER strip when grade 3 (>125 PMN/cu mm) was taken as cut off were 87.5% and 95.3% respectively with a positive predictive value of 80% and negative predictive value of 97.2%. The sensitivity and specificity of LER strip when grade 4 (>500PMN/cu mm) was taken as cut off were 53.1% and 100% respectively with a positive predictive value of 100% and negative predictive value of 90.8%.*

CONCLUSION *LER strips usage in ascitic fluid seems to be a promising tool in diagnosis of spontaneous bacterial peritonitis at the bedside. It is a rapid, cost effective diagnostic tool with a 100% positive predictive value when grade 3 and above is taken as a cut off point.*

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is potentially lethal complication of cirrhotic ascites, with a prevalence of 10% to 30% among hospitalized cirrhotic patients. Although early recognition and initiation of antibiotics produces satisfactory response in most cases, the mortality remains as high as 30-50%. Improved survival in SBP can be achieved by rapid diagnosis and prompt intiation of treatment. Presence of more than 250 polymorphonuclear leukocytes (PMNL) per microlitre of ascitic fluid is considered a surrogate marker for diagnosing SBP, irrespective of the ascitic fluid culture report and to initiate therapy (1,2). Symptoms and signs are frequently absent in patients with SBP, hence a diagnostic paracentesis should be performed in all patients with ascites admitted regardless of signs or symptoms; based on clinical suspicion. However, access for a quick total and differential leukocyte count, may not be readily available at all times, so availability of a rapid screening test at bedside will help in early diagnosis of SBP and reduce the morbidity and mortality associated with it. Leucocyte esterase reagent (LER) strips, developed initially to test for PMNL in urine, have been shown to be useful in detecting PMNL in other body fluids such as pleural fluid, cerebrospinal fluid and ascitic fluid (3,4,5,6). The aim of the present study is to study the efficacy of leucocyte esterase reagent strip in diagnosis of SBP in cirrhotic patients.

MATERIALS AND METHODS

A prospective, observational study was conducted in a tertiary care teaching hospital at Chennai from October 2012 to August 2014. 200 patients above the age of 18 years; admitted with ascites in medicine wards and Intensive care unit (ICU), confirmed by clinical and ultrasound examination were included in the study. Patients who have received antibiotics in previous 48 hours, who underwent recent abdominal surgery within the previous 3 months, and pregnant patients were excluded from the study. Detailed history and appropriate investigations were recorded in a pre structured proforma. Ascites was confirmed by USG abdomen and abdominal paracentesis was done to ascertain the cause for ascites and to rule out SBP. 10 ml fluid was seperately collected into a test tube and the LER strip (Multistix10SG) was dipped into the fluid for 30 seconds and the colour change was noted after 2 min. In this test, esterase activity of PMNL in the fluid acts on an ester substrate releasing 3-hydroxy-5-phenylpyrrole, this changes the colour of azo dye in the reagent strip. The change in colour is compared against the standard chart provided, and graded from 1 to 4 as follows.

Grade 1 - no change colour indicates - <15 PMN's/cu millilitre.

Grade 2 - light grey colour indicates - <70 PMN's/cu millilitre

Grade3 - light blue colour indicates - 125 to 500 PMN's/cu millitre

Grade4 - Purple color indicates - >500 PMN's /cumillitre.

The reading of the colour was done by a single trained observer inorder to avoid interobserver variation. The results from LER strip test were compared with the gold standard test for diagnosis of SBP the Absolute neutrophil count (ANC) more than 250 cells per cumm. Thereby the sensitivity, specificity, positive predictive value, negative predicitive value were calculated to validate efficacy of LER in diagnosing SBP at bedside.

RESULTS

A total of 200 patients with ascites were studied of whom 180 were ascites due to cirrhosis. A male predominance (89%) was noted compared to females (11%). The mean age of presentation was 50.16 years (age range being from 23 to 76 years). The most common etiology of ascites in chronic liver disease was ethanol. Table-1 shows the various etiologies of chronic liver disease among the study population.

Table-1: etiology of CLD among patients with SBP.

		GROUPS					
		SBP		NON SBP		TOTAL	
CLD ETIOLOGY	ETHANOL	21	65.6%	99	66.9%	120	66.7%
	HBV	7	21.9%	30	20.3%	37	20.6%
	HCV	2	6.3%	5	3.4%	7	3.9%
	CRYPTOGENIC	2	6.3%	11	7.4%	13	7.2%
	AUTOIMMUNE	0	0%	3	2.0%	3	1.7%
	TOTAL	32	100%	148	100%	180	100%

In our study fever was the predominant presenting symptom in 68.8%. The clinical presentations are shown in Figure-1.
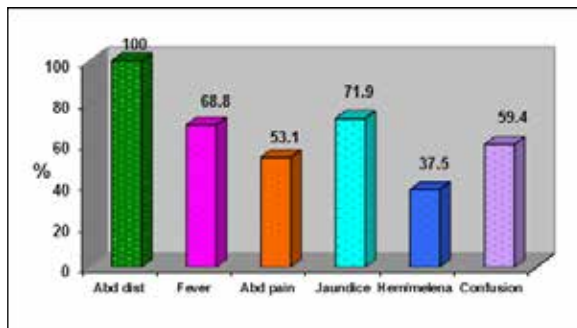

Figure-1: presenting features of patients with SBP

Among the 180 patients with cirrhotic ascites, incidence of SBP was 17.8% and it was found to be more among patients with Alcoholic liver disease (65.6%). Out of 32 cases of SBP, cultures were positive in 5 cases (15.6%). There were 2 cases of Escherichia coli, 2 cases of Klebsiella pneumonia and 1 case of Staphylococcus aureus. The sensitivity and specificity of LER strip test in diagnosing SBP when grade 3 (>125 PMN's/cumm) was taken as cut off point was 87.5% and 95.3% respectively as shown in Table-2.

Table-2:comparision betweenANC and LER grade 3

ANC				
		Positive	Negative	Total
LER	Positive	28	7	35
	%of total	15.6%	3.9%	19.4%
	Negative	4	141	145
	% of total	2.2%	78.3%	80.6%
	Total	32	148	180
	% of total	17.8%	82.2%	100%

TRUE POSITIVE	TRUE NEGATIVE	FALSE POSITIVE	FALSE NEGATIVE
28	141	7	4

Positive predictive value and negative predictive values were 80% and 97.2% respectively. The sensitivity and specifity of LER strip test in diagnosing SBP when grade 4 was taken as cut off was 53.1 % and 100% as shown in Table-3.

Table-3:comparision between ANC and LER grade 4

ANC				
		Positive	Negative	Total
LER	Positive	17	0	17
	%of total	9.4%	0%	9.4%
	Negative	15	148	145
	% of total	8.3%	82.2%	80.6%
	Total	32	148	180
	% of total	17.8%	82.2%	100%

TRUE POSITIVE	TRUE NEGATIVE	FALSE POSITIVE	FALSE NEGATIVE
17	148	0	15

Positive predictive value and Negative predictive value were 100% and 90.8%. Figure-2 shows the ROC curve and Table-4 shows the comparison of the sensitivity, specificity positive predictive and negative predictive value between the LER grade 3 and grade 4. The mortality rate in our study was 37.5% (12 out of 32) among the patients with spontaneous bacterial peritonitis.
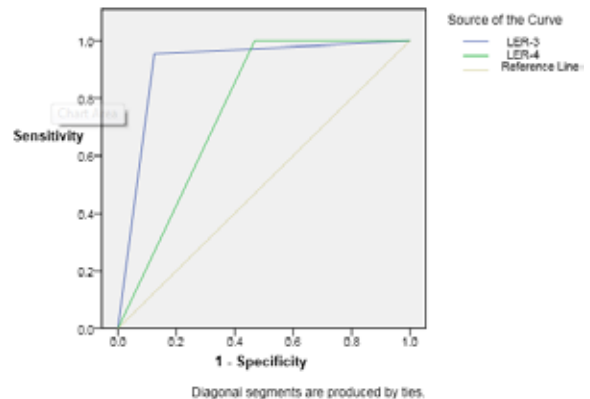

Figure-2: ROC curve

Table-4 : Comparison of sensitivity,specificity, positive predictive value and negative predictive value between grade 3 and grade 4 LER

	LER 3	LER 4
SENSITIVITY	87.5	53.1
SPECIFICITY	95.3	100
POSITIVE PREDICTIVE VALUE	80	100
NEGATIVE PREDICTIVE VALUE	97.2	90.8

DISCUSSION

Spontaneous bacterial peritonitis is the infection of ascitic fluid in the absence of hollow vicus perforation or an inflammatory focus in the abdomen like an abscess, acute pancreatitis or acute cholecystitis. The postulated mechanism for SBP is the translocation of bacteria from gut lumen to mesenteric lymph nodes or other extraintestinal sites in the presence of impaired immune defense mechanisms such as low ascitic fluid complements. These fac-

tors along with porto-systemic shunting cause a transient bacteremia, promoting ascitic fluid colonization. Moreover the decrease in small bowel motility and intestinal transit time further augment the bacterial overgrowth. Patients with SBP have a varied clinical presentation ranging from nearly 30% being asymptomatic to symptoms like fever, jaundice and abdominal pain to encephalopathy or shock. Puri AS et al reported 21 out of 70 (30%) had SBP or its variants (7). Stogaard J S et al, in contrast to most other studies, diagnosed SBP only on the basis of Ascitic fluid culture regardless of the number of WBC's. They found a 7.7% incidence, which was much higher compared to 2.7% incidence in our study (8). Luke T Evan et al reported an incidence of SBP in a population of 427 cirrhotic out patients as 3.5% (9).

Great variation in the presenting features of SBP have been reported in the previous studies (10,11). Minhas et al reported fever 54%, pain abdomen in 57% and Hepatic encephalopathy in 67% of patients (12). In another study, Pelletier et al found 89% of patients had fever, UGI bleed (42%) , pain abdomen 53% and Hepatic encephalopathy in 50% of cases (13). In our study among the presenting symptoms fever is present in 68.8%, hepatic encephalopathy in 59.4%,pain abdomen in 53.1% and gastrointestinal bleeding in 37.5% of patients. In this study, out of 32 cases of SBP, organisms were isolated in 5 cases (15.6%).

There were 2 cases of Escherichia coli, 2 cases of Klebsiella pneumonia and 1 case of Staphylococcus aureus. E. Coli was found as most common organisms in most other studies contributing for nearly 60% of all positive cultures. The other common isolates were Klebsiella pneumonia and Pneumococcus. Jain et al, found Staphylococcus as the most common organism (14) while David D et al found Streptococcus growth in 53% of the culture positive cases (15).

An early diagnosis of SBP is essential in order to overcome the complications, thereby reducing the morbidity and mortality associated with it. The presence of ANC more than 250 per cu mm is diagnostic of SBP. But at times it becomes difficult to get the counts immediately to decide on management. This mandates an alternative method at bedside to diagnose the presence of SBP. Dip stick tests for diagnosis of SBP, in cirrhotic ascites were evaluated using a reagent strip containing leukocyte esterase (LERs) which was originally designed for testing urine (16,17,18).

In the present study the sensitivity and specificity of LER strip test in diagnosing SBP when grade 3 (>125 PMN's/cumm) was taken as cut off point was 87.5% and 95.3% respectively while the sensitivity and specificity of LER strip test in diagnosing SBP when grade 4 was taken as cut off was 53.1% and 100%. In a pilot study, the reagent strip was found to have a sensitivity, specificity and negative predictive value of 89, 99 and 98% respectively. Balagopal SK et al studied 175 patients with cirrhotic ascites and reported 75 patients with SBP. Two different cut off points for calling the LER strip positive were studied, they were Grade 2 (PMNL>125 cells/cumm) and Grade 3 (PMNL>500). Sensitivity and specificity of grade 2 cutoff was 97% and 89% respectively, whereas grade 3 cutoff point showed sensitivity of 92% and specificity of 100% (19).

Butani et al used LER strip to diagnose SBP in 136 ascitic fluid specimens and found the sensitivity, specificity, PPV and NPV of the strip as 83%, 99%, 91%, 98% respectively (20). Li et al reported 25 patients of SBP demonstrated a sensitivity of 92.8% and specificity of 84.7% with the urine dipstick in ascitic fluid samples (21).

Overall, this study showed a good sensitivity and specificity of LER strip in diagnosis of SBP when grade 3 is taken as cut off point. LER strip is a simple, rapid, cost effective method of diagnosis of SBP with reasonably good accuracy.

The limitations of our study are a smaller sample size and there was no definite cut off point for the LER strip at 250 PMN's per cumm, which is the gold standard for diagnosis of SBP.

CONCLUSION

LER strip usage in ascitic fluid seems a valid option in providing a rough estimate of the absolute neutrophil counts in the fluid. Promising results were found when grade 3 reading on the strip was taken as the cut off point with a good sensitivity, specificity, positive predictive value and negative predictive value. Hence LER strip can be used as a rapid, bedside tool for diagnosis of SBP.

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