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Original Research Article

Use of Leucocyte Esterase Activity in Urinary Dipstick Test for Diagnosis of Spontaneous Bacterial Peritonitis

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Conflict of interest: Nil

Abstract:

Background: Spontaneous bacterial peritonitis (SBP) is a life-threatening complication of ascites and needs rapid diagnosis and initiation of antibiotics. Diagnosis of SBP employs cytobacteriological analysis of ascitic fluid which requires good laboratory facilities that can take few hours to 1–2 days to report the results. 24 hr laboratory facilities are not widely available in India. We assessed the utility of the reagent strip (Multistix 10 SG®) for rapid diagnosis of SBP.

Material and Methods: Ours was a prospective, cross-sectional diagnostic accuracy study carried out on patients with cirrhosis of liver with ascites admitted in a tertiary medical college. Multistix 10SG reagent strip test was administered on the ascitic fluid. Cell count was determined by colorimetric scale of reagent strip and was compared with the counting chamber method. Sensitivity, specificity, positive and negative predictive values were evaluated for the reagent strips.

Result: Of the 100 cirrhotic patients with ascites, [7 females:93 Males] 52 subjects were diagnosed with SBP by the counting chamber method; as compared to 51 patients detected to have SBP by Multistix 10 SG reagent strip test (3+ positive). In comparison to conventional counting chamber, Method reagent strip 3+ had sensitivity, specificity, positive and negative predictive values 98%,100%, 100% and 97.9%.

Conclusion: Multistix 10SG when compared to counting chamber method is very specific and sensitive in diagnosing SBP. It is a fast and convenient diagnostic tool that gives the result in 2 minutes and permits rapid initiation of antibiotic therapy.

Introduction

Spontaneous bacterial peritonitis (SBP) is the most common life threatening (30%-50%) complications of ascites[1]. SBP refers to the infection of the ascitic fluid in absence of any intra-abdominal source of infection (intraabdominal abcess, intestinal perforation)[1,2] or inflammation like pancreatitis, cholecystitis etc [2]. SBP is suspected whenever there is a clinical deterioration in a case of cirrhosis. The mechanism most commonly implicated is the activation of Renin Angiotensin Aldosterone System (RAAS) secondary to splanchnic vasodilatation which occurs due to accumulation of vasodilatory substances (Nitric Oxide) leads to retention of fluid and development of ascitis. This is accompanied by migration of intestinal organisms across the mucosa to the extraintestinal sites and blood leading to SBP and systemic infection [3]. The patient may present in encephalopathy and with jaundice or the ascites may be come painful [4,5]. The current gold standard for the diagnosis of SBP is establishing a PMN count >250cells/mm^3 or the ascitic fluid culture being positive for bacterial growth [6]. Positive diagnosis for SBP is followed by the initiation of antibiotic therapy, 3rd generation cephalosporins being the current gold standard [7]. In the institutes where 24-hour labs are available the results of the PMN count in the ascitic fluid can be at the earliest obtained in an hour, the results of culture however take 48 hours to be reported [7]. In Primary Health centres where 24 hour labs are not available the results of PMN can

take a day or more to come and thus delaying the initiation of therapy.

LERS (Leukocyte Esterase reagent strips) are used for the diagnosis of (UTI) Urinary tract infection [8]. The multistix 10 SG used for the diagnosis of UTI asseses 10 parameters like Leukocyte count, RBC count, bilirubin levels etc in the urine. It gives all these results within 2 minutes of contact with the urine sample. Each parameter is represented by a different colored strip. The degree colour change in each strip represents the magnitude of each parameter [9]. Making use of this property LERS have been used for the diagnosis infection in the synovial, cerebrospinal fluid etc [9]. As the results are obtained within 2 minutes, a decision to start the antibiotic therapy can be taken without waiting for the definitive lab results. Use of LERS for diagnosis of SBP is of importance more so in the PHC's and remote areas where early results of the ascitic fluid PMN count are not available. Our study aims at comparing the LERS to the current gold standard methods for early initiation of antimicrobial therapy.

Among the earlier studies was a study done in an American and a European centre by sapey et al [1]. This study took 245 samples from 51 patients. On comparison of the dipstix with the ascitic fluid counter result a sensitivity of 64.7%, specificity of 99.6%, PPV of 91.7 and NPV of 97.4 was obtained [8]. The first study that used Multistix strips for the diagnosis of SBP was done in United States in 2000 [10]. This study took 136 cirrhotics and used the dipstick test and simultaneously studied the ascetic fluid samples for total and differential count and the cultures [10]. A high sensitivity and specificity of 83%, 99% with a positive and negative predictive value of 91% & 98% respectively [10] was derived in this study.

Since then many publications have followed. The first study that validated the Multistix ®8SG was of Vanbiervliet et al [11]. Most of studies with the exception a French multicentre study (Nousbaum et al [12], 70 centres) showed promising results. Castelote et al [13] showed that despite the qualitative nature of LERS, could be useful in the diagnosis of SBP. The low cost of the strips, in a country like India, has significant advantage. Multiple brands of LERS are available and there are multiple studies in which some of these brands have been used for the diagnosis of SBP. Similarly other studies done internationally showed a high sensitivity and specificity of LERS [16,18-20].

Among the studies done in India one was done in RNT medical college Udaipur [14]. In this study 100 cases of cirrhosis were taken. They were tested for SBP using Multistix 10 SG. In this study the results of the dipstix were compared with the counting chamber [14]. This study revealed 77.17% sensitivity, 95.12% specificity, 95.12% positive and 92% negative predictive value.

Among the recent ones done in India is a single centre study done in PGIMS Rohtak [15] where 103 patients with cirrhosis of liver were taken. The Multistix 10 SG was then used in the ascitic fluid samples. Out of the 103 patients, diagnosis of SBP was made in 20 patients by manual cell count. The sensitivity, specificity, positive and negative predictive values were reported as 95%, 96.4%, 86.4% and 98.8% [15].

Since many studies have been done on Multistick 10 SG no research gaps were found to be solved in this study.

Methods

The study was carried out over a span of two years from September 2018 to October 2020 at a tertiary care Medical College in South India under the guidance of Department of General Medicine with assistance from the Department of Gastroenterology. This study was approved by the ethical committee.

Men and women with chronic liver disease and ascitis were approached for the study. Patients with any history of gastro-intestinal surgical procedure in the preceding 4 weeks and presence of condition that lead to increase in neutrophil counts in ascetic fluid (like pancreatitis, pancreatic carcinoma, tuberculosis) were excluded from the study. In our study, finally, a total of 100 cases, both male and females, of cirrhotic ascites admitted under the department of general medicine and gastroenterology were included after a written informed consent.

Data Collection

Once the suitable cases had been identified the individual profile of each case was recorded and a detailed history was taken. Each participant was then examined and the relevant findings were recorded. In each case abdominal paracentesis was done under aseptic precautions and the samples were taken for Multistix 10 SG testing and also sent for calculation of the total and differential counts. The samples were simultaneously drawn and sent for culture studies too.

For the Multistix 10 SG testing the dipstix was dipped in the ascitic fluid sample such that the colorimetric strip used for the calculation of poly-morpho-nuclear (PMN) cell count was in contact with the ascitic fluid. The strips were then withdrawn and read after 2 minutes to look for the colour change. The colour change was recorded and the results were recorded as 0, trace, 1+, 2+, 3+ signifying 0, >15, >70, >125 and >500 PMN cells respectively. The results of the total and differential cell count in the ascitic fluid sample were then

compared with the results of Mulltistix 10 SG strips.

Statistical Methods

Data Analysis: Data analysis was done using MS Excel for data entry and SPSS v20 for further analysis. Validity parameters namely sensitivity, specificity & predictive values of positive and negative & accuracy of urinary dipstick test with respect to PMN count & ascitic fluid culture has been computed.

Results

100 cases of cirrhotic ascites fulfilling the inclusion & exclusion criteria were studied and analyzed. Ninety-three of the cases were males and the rest were females. The cause of cirrhosis was investigated in detail and documented.

Ethanol intake accounted for the majority of the cirrhotic patients (62 in number), followed by hepatitis B & C (12 cases). One case was attributed to have non-alcoholic steato-hepatitis and one was found to have hemo-chromatosis.

No etiology could be ascertained in 19 of the patients and these were classified as cirrhosis with a cryptogenic cause (Table 1).

Table 1: Etiological details of the cases

Ethanol	62 (62%)
Hepatitis B & C	12 (12%)
Hereditory hemochromatosis	1 (1%)
Amyloidosis	1 (1%)
NASH	5 (5%)
Cryptogenic	19 (19%)

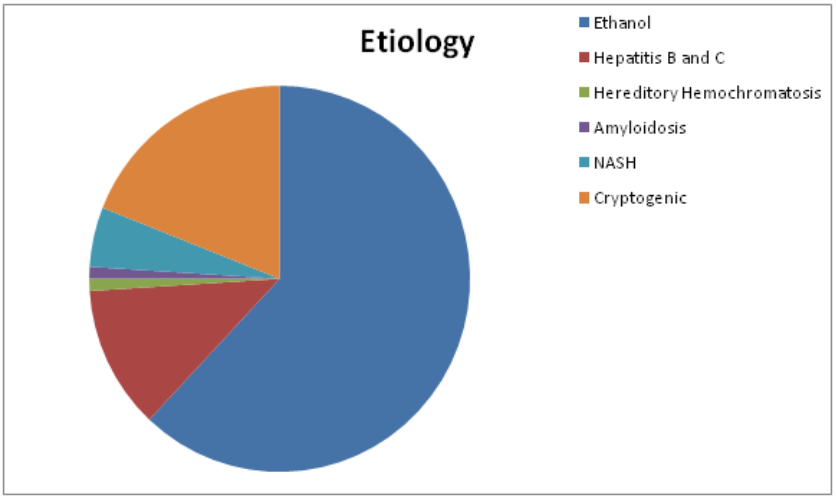

Figure 1:

Spontaneous bacterial peritonitis was diagnosed based on the differential count in the drawn samples. The samples were also sent for total counts, culture studies and LERS testing. Fifty two patients were found to have spontaneous bacterial peritonitis. Among these, 46 were males and the rest were females (Table 2).

Table 2: Cases with and without spontaneous bacterial peritonitis among both sexes as per the lab values (Differential count)

	SBP [Number (%)]	**Non- SBP[Number (%)]**
Males	46 (88.4%)	47 (97.9%)
Females	6 (11.5%)	1 (2.1%)
Total	52 (100%)	48 (100%)

Six samples out of 100 grew micro-organisms on cultures. These were among the 52 which were found to have spontaenous bacterial peritonitis on initial testing. Three of the samples grew E. coli, 1 grew Klebsiella, 1 grew a mixture of enterobacter & klebsiella and 1 grew only enterococcus (Table3).

Table 3: Culture results

Results of the culture	**Number / %**
Culture positive	6
Culture negative	94
Total number of cases	100

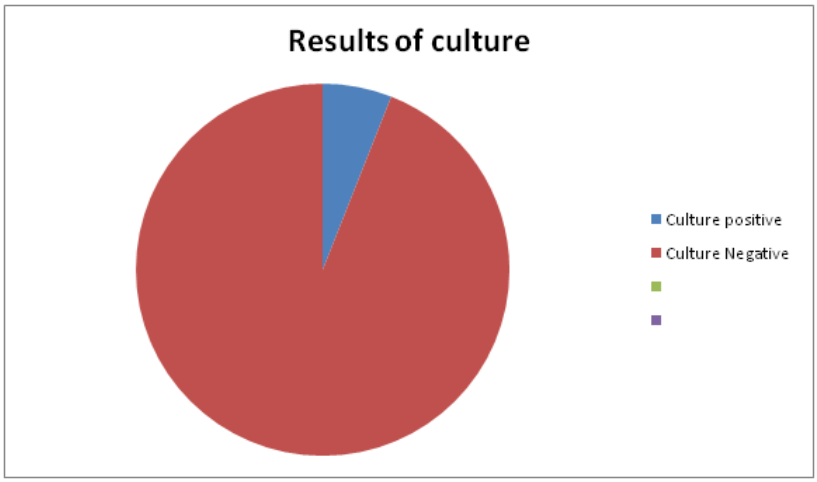

Figure 2:

As per the multistix 10SG testing, a cell count in excess of 500 polymorphonuclear cells was suggestive of spontaenous bacterial peritonitis. Fifty one cases were positive for SBP as per this criteria. Three cases had >125 neutrophils, 10 had >75 neutrophils, 6 had trace cells and 30 had no cells.

Table 4: Results of Multistix 10 SG

Results of the Multistix 10SG for all samples	Number	Percentage
0	30	30%
Trace	6	6 %
1+	10	10%
2+	3	3%
3+	51	51%
Total	100	100%

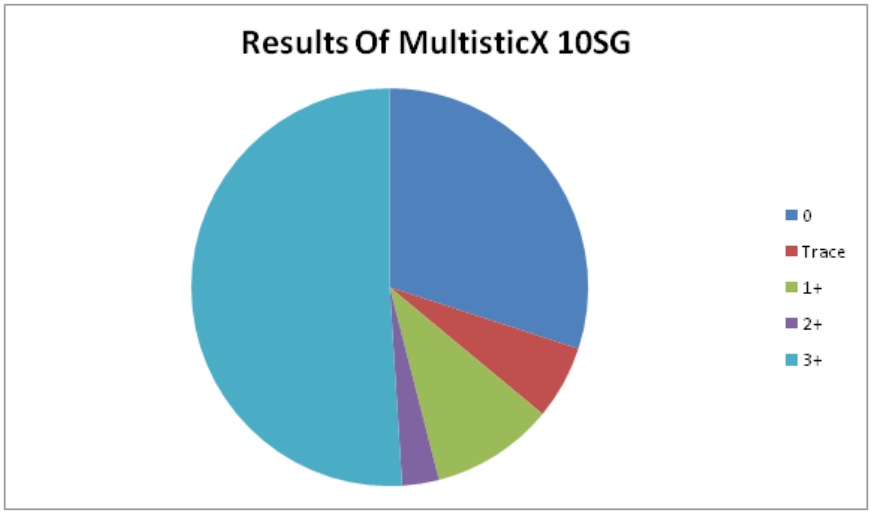

Figure 3:

Analysis

The results were analysed and the Multistix 10SG dipstick was found to have sensitivity 98% and specificity 100%. The positive (PPV) and negative predictive values (NPV) were 100 and 97.9% respectively. The P value, calculated as per the Mc Nemar's test was found to be 0.98. This implies that, there is minimal disagreement between the results of the Gold standard test and Multistix 10SG test. This concludes that the agreement between the two tests is significant statistically.

Ethical approval

Written informed consent which was printed in either Vernacular Language or English was taken from the patients prior to the interview.

The study was undertaken after ethical approval by institutional ethics committee.

Discussion

Cirrhosis of liver is a disease widely prevalent in our country. There are a number of etiologies for the

same. As per our study, ethanol consumption was found to be the most common etiology (62 patients), this highlights the fact that ethanol consumption continues to be the major contributor to the cirrhotic load in the country. With the total counts samples had been also sent for the culture studies. Our study revealed 6 samples to grow micro-organisms, 3 of which grew E. coli, this validates the fact that continues to be amongst the most common organisms implicated. Once the cirrhosis develops it often leads to the development of different complications. Among the well-known and lethal complication of cirrhosis is Spontaneous Bacterial Peritonitis. Rapid diagnosis for initiation of antibiotic therapy is the cornerstones in the management of SBP. These groups have a low –40% culture yield with 48 hours being the time taken to report the culture. Thus initiation of antibiotics is usually done on basis of >250/mm3 PMN count [9].

However, AF manual cell counting is not easily available in all hospitals, especially in cases of emergency. Reporting even with a good lab takes about an hour and in absence of a 24 hour on-call pathologist may be delayed even by a day, there can also be a leukocyte clump formation that gives spurious results.

Recently, reagent strips have emerged as a simple, fast and attractive means for rapid diagnosis of SBP. They were able to detect esterase activity of PMN cells. Multiple independent studies have evaluated the diagnostic importance of LERS (Multistix_, Combur_, Nephur_, UriScan_ and Aution_ strips) in cases of SBP. The earlier published studies have shown widely variable results in terms of the sensitivity, the specificity, the PPV and the NPV; compared to manual PMN count (which is the gold standard), the LERS were found to have range of sensitivity between 45 to 100%, specificity between 81 to 100%, PPV value between 42 to 100% and NPV value from 87 to 100%. [9-20]. Therefore, previous studies which used Multistix reagent strips, showed widely variable test results.

A study was done in RNT medical college Udaipur [14]. In this study 100 cases of cirrhosis were taken. They were tested for SBP using Multistix 10 SG. In this study the results of the dipstix were compared with the counting chamber [14]. This study revealed a sensitivity of 77.17%, specificity of 95.12% , positive and negative predictive values of 95.12% and 92% respectively.

A single centre study was done in PGIMS Rohtak [15] where 103 patients who had been diagnosed with cirrhosis of liver were taken. The Multistix 10 SG was then used in the ascitic fluid samples. Out of the 103 patients SBP was diagnosed in 20 patients by manual cell count. The sensitivity and specificity of this study was reported as 95% and 96.4% resp. with the positive and negative predictive value being reported as 86.4% and 98.8% [15].

Our study showed a sensitivity of 98% and a specificity of 100% with positive and negative predictive values of 100% and 97.9% respectively. In some of the mentioned studies multiple samples were taken from the same patient [18,19] Taking samples from the same patients cannot effectively rule out the effect of antibiotics that might have been previously used. In our study a single sample was taken from 1 patient for analysis ones. This study explores the earlier findings of the utility of LERS test as a rapid, simple and inexpensive means for testing of SBP. There is a wide variation in the sensitivity and PPV between various studies, few studies have raised doubt over the use of LERS as a valid surrogate marker for SBP[14].

However, because of consistently excellent NPV (>95% in majority of the studies) of LERS, others have advocated its place in the ascitic tap diagnostic algorithm, especially as a preliminary screening tool for diagnosing cases SBP. With the use of bedside LERS test, early treatment with antibiotics can be initiated in asymptomatic SBP patients. Also it is cheaper than the manual cell count.

Though a number of international studies have been done, sufficient studies on use of LERS in India are lacking. Our study was the first in Southern India. However more studies with a greater number of patient enrolments are warranted.

Limitations

Limitation of our study is that it had only 7 female patients, also that ours is as a tertiary care centre and with most of the patients being referred the study population cannot be considered representative of the whole population. Another Limitation of this study is that, the Dipstick results were interpreted by a single observer. There is always a possibility of inter-observer variation in the matching of colour.

Conclusion

Summarizing this LERS can be used as a cheap and reliable tool to diagnose SBP early and can be used for initiation of antibiotic therapy without delay. Also, as 24 hour good laboratory facilities are not available in many remote areas of the country LERS can be used at least to initiate the antibiotic therapy with the further management to be planned once the cytobacteriological results are available.

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