Utility of Leukocyte Esterase Dipstick test in bed side diagnosis of Spontaneous Bacterial Peritonitis in Patients of Cirrhosis of Liver with Ascites

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Research Article

Abstract: **Background**: Spontaneous bacterial peritonitis (SBP) is commonest and severe complication of decompensated cirrhosis. SBP is defined as the infection of ascitic fluid (AF) in the absence of a contiguous source of infection and/or an intra-abdominal inflammatory focus. An AF polymorphonuclear (PMN) leucocyte count ≥ 250/mm3 -irrespective of the AF culture result- is universally accepted nowadays as the best surrogate marker for diagnosing SBP. 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. **Aim**: Objective of our study was to find out the diagnostic accuracy of leukocyte esterase dipstick test for the diagnosis of spontaneous bacterial peritonitis. **Methods**: This cross-sectional study was conducted from January 2013 to August 2013 at a tertiary care center in central India . All the Patients with cirrhosis and ascites of either gender were included in this study. A total of 104 patients were enrolled for the study. Patients with secondary bacterial peritonitis and those who have received antibiotic therapy during past 10 days of hospital admission were excluded from the study. All the patients underwent abdominal paracentesis. Urine dipstick (Piramal10x test-India) was used as screening test and the results were compared with manual cell counting and ascitic fluid culture. A cut-off of 4+ on dipstick test was considered as positive for SBP. **Results**: The manual cell count using the above criterion diagnosed 28.9% patients as having SBP. The dipstick results were compared with the PMN counts. Sensitivity, Specificity, Positive predictive value, Negative predictive value and accuracy was 83.3%, 95.9%, 89.3%, 93.4% and 92.3% respectively.
Conclusion: Leukocyte esterase dipstick test emerged out as an effective tool for rapid, bedside diagnosis of SBP.
Keywords: spontaneous bacterial peritonitis, ascitic fluid, polymorphonuclear.

Introduction

Spontaneous bacterial peritonitis (SBP) is one of the most important complications of liver cirrhosis with ascites and is characterized by spontaneous infection of the ascitic fluid without an intraabdominal source of infection. The prevalence of SBP varies from 3.5 to 30% depending on the population examined (Outpatients or hospitalized)[1]. Early diagnosis and treatment of SBP is essential for survival of the patients[2,3,4]. Most commonly used method for diagnosis of SBP in clinical practice is demonstration of ascitic fluid polymorphonuclear count (PMN). PMN count of ≥ 250 per µl irrespective of ascitic fluid culture result is universally accepted nowadays as the best surrogate marker for diagnosis of SBP[1,5]. However, diagnosis of SBP takes hours by cell counter method. Also, the laboratory back up that is required for rapid diagnosis of SBP is not available everywhere and specially in the rural set ups. Ascitic fluid culture also takes few days for precise diagnosis of SBP. Due to time consuming nature of currently available diagnostic tools, there is often a delay in diagnosis and treatment of SBP. And this presses for the need for rapid diagnostic test of SBP. Use of leukocyte esterase (LE) dipstick test is supposed to reduce the time required for presumptive diagnosis of SBP from hours to minutes. Originally, LE dipsticks were designed for diagnosis of urinary tract infections. Recently, it has also been used for rapid diagnosis of infection in body fluids such as plural fluid, CSF etc.[4,6,7]. Recently, many studies have shown the efficacy of dipstick in diagnosing SBP. Basic principle behind LE dipstick test is that leukocyte esterase released from PMN cells react with esterified compound in the reagent strip yielding a violet azo dye[4,8]. Through this study, we intend to find out the diagnostic utility of leukocyte esterase dipstick test in rapid and bedside diagnosis of SBP in patients of cirrhosis of liver with ascites.

Materials and Methods

In view of the above objective, a single centered hospital-based cross sectional study was planned and conducted during January 2013 to August 2013 at NKP SIMS and Research Center, Nagpur which is one of the

tertiary care units in Central India. Study was conducted on approval by the institutional ethical committee. Informed consent of all patients who were recruited for this study was taken. All patients with diagnosis of liver cirrhosis with ascites of age more than 18 yrs; of either sex were included in the present study. Patients who have received antibiotic therapy during past 10 days of hospital admission or at home for any reason, patients with history of any surgical procedure in the previous four weeks, tuberculous ascites, pancreatic and malignant ascites were excluded from the study. A total of 130 patients were screened during the study period. Of these, 104 patients were eligible as per the inclusion and exclusion criteria. All patients underwent abdominal paracentesis under all aseptic precautions. Multistix 10 SG dipstick was used as the screening test and the results were compared with the manual cell counter and ascitic fluid culture. A cut off of ++++ which corresponded with 500 leukocytes/ cubic mm on dipstick test was considered as positive for SBP and the results were read at one minute. At least 10 ml ascitic fluid was collected in blood culture bottle and inoculated on aerobic as well as anaerobic media.

Statistical Analysis

Ascitic fluid samples were analyzed by using cell counter and dipstick test. Also the cell culture data was obtained for each sample. The diagnosis of SBP was done by using cell counter criterion and the results were compared with the dipstick results. Accordingly, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the test was determined. Also, the results of dipstick test were compared with those obtained through cell culture.

Results

One hundred four ascitic fluid samples were considered for the study. The mean age of selected patients was 45.77 ± 14.62 years, with male to female ratio of 3:1 (Table 1). The most common etiology for liver cirrhosis was alcohol (74%) followed by Hepatitis B (8.65%). We did not get any patient with Hepatitis C in the study. Thirty of the 104 patients were SBP positive according to cell counter criterion. Out of these SBP positive cases, 25 patients also showed positive result on dipstick as shown in Table 2. Referring PMN as a standard, the sensitivity of dipstick was obtained as 83.33%, specificity as 95.9%, PPV as 89.3% and NPV as 93.4%. The accuracy of the test was 92.3%. The Cohen's kappa value as an indicator of agreement between two tests was obtained as 0.8088 [95% CI: 0.6815 – 0.9361], suggesting that there is *almost perfect agreement* between the results of manual cell counter and Leukocyte esterase dipstick test. These results were comparable with the results from different studies from abroad as well as from India. The samples were also subjected to microbiological culture analysis and the results were compared with that of the Dipstick test (Table 3). The comparison resulted into a sensitivity of 66.6%, specificity of 79.7%, PPV of 35.71% and NPV of 93.4%. The overall agreement between the two was 77.88%. The Cohen's Kappa statistic obtained was 0.3434 [95% CI: 0.1039 – 0.5789] indicating a *fair agreement*. The culture details for fifteen cases along with cell count and Dipstick test results are shown in Table 4. It reveals that *E. coli* is the most dominant organism in the studied samples. Further, table shows that results of leukocyte esterase dipstick test were correlating strongly with the cell counter results; irrespective of the culture results. There was only one patient of monomicrobial non-neutrocytic ascites with pseudomonas growth in culture in whom LE dipstick test was also positive.

Discussion

SBP continues to be an important source of morbidity and mortality in patients with cirrhosis[9,10]. In such circumstances, prompt diagnosis and treatment of SBP are crucial to ensure better clinical outcomes in this patient group. Manual cell counter takes hours for diagnosis of SBP. Also, the laboratory back up required for diagnosing SBP is not available readily in small set ups, especially in rural areas. It has been shown that positive bacterial culture is obtained in just 40% patients with SBP[4,9]. Diagnosis can be further delayed if the results are dependent solely on culture reports. In many parts of the world as well as in India, the utility of LE dipstick test in diagnosing SBP has been confirmed[11-16] (Table 5). The overall sensitivity, specificity, PPV and NPV are close to 90%. Our study also supports the use of LE dipstick test in rapid bedside diagnosis of SBP and the results are comparable with the results of the above studies. However, the correlation between the culture results and the dipstick test was poor. Six out of 15 samples (40%) indicated positive cultures but were negative as per the test. Some of the advantages of LE dipsticks are that they are easily available even in the local markets and economically cheaper as compared to cell counter and culture. Less expertise is required to diagnose SBP based on dipsticks. However, there are some practical problems with the usage of LE dipstick test as well. At least till date, there is no commercially available ascitic fluid specific dipstick[4]. There can be inter-observer variability in interpretation of LE dipstick results. There is no standardized cut-off value on the colorimetric scale. Usage of different dipsticks can bring variation in results. Precise estimation of the severity of infection is not possible with LE dipstick as it provides only qualitative assessment of the presence or absence of ascetic fluid infection. Limitation of this study is that the analysis of SBP variants such as mono-microbial ascites,

culture negative neutrocytic ascites (CNNA), classical SBP is not done in the present study. The delays in the diagnosis of SBP are frequent, mostly in hospitals with limited laboratory facilities and in rural set up taking a huge toll on life expectancy of cirrhotic patients. Considering the results from our and other studies, LE dipstick can be a feasible option for faster and cheaper diagnosis of SBP. To conclude, LE dipstick test could be an effective tool for simple, rapid, cost effective and bedside diagnosis of SBP with considerably good amount of sensitivity, specificity, PPV, NPV and accuracy. If further validation studies worldwide are supportive, it is set to become the mainstream process of handling the ascetic fluid samples.

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Table 1: Descriptive statistics for demographic and etiological factors (n=104)

Characteristics	**Mean ± SD / *n* (%)**
Age (years)	45.77 ± 14.62
Male / Female	80 / 24
Etiology of cirrhosis	
Alcohol	77 (74.03%)
Hepatitis B	9 (8.65%)
Hepatitis C	0 (0%)
Others	18 (17.3%)

Table 2: Concordance between Dipstick test and PMC count results

Leukocyte esterase test	**PMN count**		**Total**
	□ 250/ mm^3	**< 250/ mm^3**	
Positive	25	3	28
Negative	5	71	76
Total	30	74	104

Table 3: Concordance between Dipstick and Cell culture results

Leukocyte esterase test	Culture result		Total
	Positive	Negative	
Positive	10	18	28
Negative	5	71	76
Total	15	89	104

Table 4: Culture results in studied cases and their corresponding PMN and Dipstick results

Culture	PMN cells/mL	Leukocyte esterase test
Pseudomonas	128	Negative
E. Coli	300	Positive - 500
E. Coli	360	Positive - 500
Pseudomonas	375	Positive - 500
E. Coli	280	Positive - 125
Pseudomonas	600	Positive - 500
E. Coli	2250	Negative
Pseudomonas	225	Positive - 125
E. Coli	360	Positive - 500
E. Coli	1050	Positive - 500
Klebsiella	200	Negative - SG - 1.005
E. Coli	700	Positive - 500 - SG - 1.010
Citrobacter	20	Negative - SG - 1.010
Non remember growth	60	Negative - SG - 1.005
Klebsiella	10	Negative - SG - 1.010

Table 5: A comparative evaluation of leukocyte esterase dipstix from different studies

Authors	Castellote et al	Kim et al	Butani et al	Sapey et al	Thévenot et al	Jha et al	Present study
Dipsticks	Aution sticks	UriSCAN/Mutistix10SG	Multistix®10SG	Nephur test / Multistix10SG	Multistix 8SG/Combur2test	Multistix 10SG	Multistix 10QDX
Inclusion scale	≥ 3 (PMN ≥ 250 mm^3)	≥ 3 (PMN ≥ 500/mm^3)	≥ 2 (PMN ≥ 70/mL mm^3)	N/A	≥ 3 (PMN ≥ 125/mm^3)	3+	4+
		/ ≥ 3 (PMN ≥ 75/mm^3)			/≥ 2 (PMN ≥ 75/mm^3)		
Sensitivity (%)	89	67/50	89	88/65	89	66	83
Specificity (%)	99	100/100	99	100/100	100	100	95
PPV (%)	98	100/100	89	94/92	100	100	89
NPV (%)	97	89/87	99	99/97	99	93	93