Original Article

Role of Dipstick (Leucocyte Esterase Reagent Strips) in the Rapid Bedside Diagnosis of Spontaneous Bacterial Peritonitis

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ABSTRACT

Objectives: To evaluate the dipstick in the rapid bedside diagnosis of spontaneous bacterial peritonitis (SBP) and avoid delay in initiation of antibiotic therapy and evaluate the prevalence of SBP by a conventional method.

Methods: A prospective analysis was done on inpatients and OPD patients with cirrhotic ascites at Jaipur Golden Hospital Delhi, India. The study included 110 consecutive patients with cirrhotic ascites. The patients were divided into 2 groups those with the polymorph nuclear leucocytes (PMNs) >250/mm^3 on cell count (positive result) and those with PMNs <250/mm^3 (Negative results) and were then compared to the dipstick results. The results were used to find the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the rapid dipstick method for the diagnosis of SBP. In addition, the data was used to calculate the prevalence of SBP in our hospital.

Results: A total number of 110 patients with cirrhotic ascites were studied who were presented to the Jaipur Golden Hospital, New Delhi, India with the complaint of abdominal distension. A total of 20 patients were diagnosed with SBP by the manual cell count method which is considered a gold standard for the diagnosis of SBP, and 90 patients were diagnosed as non-SBP. We re-diagnosed these 20 patients with SBP with a dipstick method, the dipstick method showed a positive result for 18 patients (90%) and a negative result for 2 patients (10%).

Conclusion: In the SBP group, the dipstick showed 18 true positives and 2 false negatives. In the non-SBP group of 90 patients, the dipstick method showed negative results in all the patients with no positive results. Thus, the dipstick showed 100% true negatives with 0% false positives. The prevalence of SBP in patients with cirrhotic ascites in a hospital was found at 18.2%.

Keywords: Spontaneous Bacterial Peritonitis, Cirrhosis, Polymorphonuclear Leucocytes, Dipstick

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is defined as the infection of ascitic fluid (AF) in the absence of a contiguous source of infection and /or an intraabdominal inflammatory focus. It is a common complication of portal hypertensive ascites in patients with either alcohol-related or nonalcoholic liver disease.[1] It occurs infrequently in patients with no cirrhotic ascites such as nephrogenic[2] and cardiogenic

ascites.[3] For SBP diagnosis, the number of polymorphonuclear leukocytes (PMN) from the ascitic fluid obtained by paracentesis must exceed 250 cells/mm^{3}. Two important things in the case of SBP are the early diagnosis of SBP and prompt initiation of antibiotic therapy. Diagnosis is usually delayed due to the delay in getting the reports from the laboratory especially out of hours. It would therefore be desirable to have a simple method of making an early diagnosis for the initiation of the treatment. Different types of dipsticks are available, and several dipsticks have been used in different studies.

SBP in patients with cirrhosis is the main consequence of bacterial translocation (BT) and this bacterial translocation is due to intestinal bacterial overgrowth because of decreased intestinal motility which is due to sympathoadrenal stimulation, increased nitric oxide synthesis, and oxidative stress of mucosa.[4-8]

The gram-negative bacteria are largely responsible for the SBP and the most common organisms responsible for E. coli, streptococci, and klebsiella. Anaerobes cause approximately 1% of SBP.[9]

Common clinical features are abdominal pain, fever, encephalopathy, and abdominal tenderness. Approximately 10% of the patients present with worsening encephalopathy without the other findings. In advanced cases, there may be paralytic ileus, hypotension, or hypothermia.[10] A significant number of patients however do not have the classical features and may present with worsening ascites or abdominal pain in the setting of CLD (chronic liver disease). A high index of suspicion and appropriate investigation is required to make the diagnosis in such cases. Diagnosis is made by the clinical features, paracentesis and analysis of ascetic fluid, ascetic fluid culture, leukocytosis, positive blood culture, acidosis, and deranged renal function. Although the ascitic fluid culture is confirmatory the single most path gnomic feature for making the diagnosis is ascetic fluid PMNs/ microliter >250. Ascitic fluid culture is negative in up to 60% of patients with SBP.

Management includes antibiotics to be given as soon as neutrocytic ascites are identified. Empirical therapy for SBP should be initiated with a third-generation cephalosporin such as cefotaxime (2 gm intravenously every 8-12 hrs depending on the renal function for a minimum of 5 days), which covers 98% of the causative agents of this disorder.[11] A similar third-generation cephalosporin, ceftriaxone 2 gm intravenously daily is considered a reasonable choice for suspected SBP in empiric therapy, while the result of ascitic fluid culture is not known.[12] If enterococcus infection is suspected, ampicillin may be added. A course of 5 days is sufficient for most patients. Ofloxacin, an oral quinolone has been demonstrated to be as effective as intravenous cefotaxime in the treatment of uncomplicated patients with SBP, but the only drawback of this is the recent observation of the emergence of the quinolone-resistant organism.[13]

Bedside reagent dipsticks

An ascitic fluid (AF) polymorphonuclear (PMN) leukocyte count > or equal to 250/mm^{3} irrespective of the AF culture result, is universally accepted nowadays as the best surrogate marker for diagnosing SBP. The presence of positive AF cultures is confirmatory but by no means a prerequisite for initiation of antibiotic therapy. Frequently the results of the manual PMN count do not reach the hands of the medical personnel in a timely manner.[14] Furthermore, manual AF PMN counting is laborious and costly. Therefore, any alternative test that may provide or, more importantly, exclude a diagnosis of SBP at the bedside and reduce the 'tap-to-first shot' time is considered desirable. So, the dipsticks commonly used in everyday practice for the rapid diagnosis of urinary tract infections (UTIs) were evaluated for the rapid bedside diagnosis of SBP. These dipsticks detect blood, bilirubin, urobilinogen, ketones, protein, nitrite, glucose, specific gravity, granulocytic leucocytes, and ascorbic acid and have been successfully evaluated in the diagnosis of infection in other sterile body fluids i.e., synovial, pleural, and cerebrospinal fluid.[15, 16]

MATERIALS AND METHODS

In this study, we evaluated 110 consecutive samples of ascitic fluid from cirrhotic patients (both admitted patients and OPD patients) in Jaipur Golden Hospital, New Delhi, India during the period from May 2015 to December 2016. The authors obtained appropriate ethical approval for this study. The diagnosis of liver cirrhosis was based on clinical, laboratory, and ultrasonographic findings.

According to our routine clinical practice, a detailed medical history, a complete physical examination, standard laboratory tests (including a complete blood cell count, prothrombin time, biochemical tests of liver and kidney function, and fresh urine sediment), a chest x-ray film, a diagnostic paracentesis, and an ascitic fluid culture were performed in all the cirrhotic patients with ascites on the day of admission and whenever they developed symptoms and signs suspicious for SBP (i.e. fever, change in mental status, abdominal pain, leukocytosis, increasing ascites development of renal failure, hypotension, *etc.*) during hospitalization. Ascitic fluid was examined for the leucocyte and the PMN cell counts /microliter. The sample for PMN and total leucocyte count was collected in a heparin anticoagulant tube. Differential cell count and the cytology were examined with a conventional optical microscope. A manual cell count with the differential study was performed for all the samples by the laboratory. Each sample of the ascitic fluid was tested using a dipstick (SD Uro color 10) which was put in an automatic cell blood counter (urometer) and the readings were taken in the form of printed reports. Those patients with a diagnosis of SBP (when the PMN cell in the ascitic fluid was greater than 250 cells/mm^3) were further analyzed.

Dipsticks are the plastic strips to which are attached several separate reagent areas. It is immersed in 5 ml of ascitic fluid placed on a dry and clean container and removed immediately to avoid dissolving out reagents. Then it is put on its side for 1-2 seconds to avoid mixing the reagents and is put after 60 seconds in the urometer. After the dipstick has been accepted by the urometer, it is measured by means of reflectance photometry. The results are automatically calculated and printed on the report forms. Urometer is a portable bedside electronic device that is based on reflectance photometry.

Diagnostic criteria

PMNs >250/mm^3 in ascitic fluid irrespective of a positive ascitic fluid culture and clinical signs of SBP and an absence of an intra-abdominal source of infection is diagnostic of SBP.

Principle

It is based on the granulocytic leucocyte esterase activity thereby detecting granulocytes in the sample. A pyrrole, esterified with an amino acid is used as the substrate; hydrolysis of the ester (mediated by the esterase) releases the pyrrole which in turn reacts with a diazonium salt yielding a violet or purple azo dye in the relevant pad of a strip.[16]

Advantages of Dipsticks

Rapid bedside diagnosis, Simplicity, Cheap (one dipstick costs approx. 15 rupees), Can be widely used in health care centers with no laboratory facilities, and can be used during off working hours (5 pm to 9 am) were due to the storage of sample till morning leads to the cell degeneration resulting in the false results.

Clinical Evaluation

The patients were divided into 2 groups those with the PMNs >250/mm^3 on cell count (positive result) and those with PMNs <250/mm^3 (negative results) and were then compared to the dipstick results.

Statistical Method

Statistical analysis was performed by the SPSS program for Windows, version 10.1 (SPSS, Chicago, Illinois). Continuous variables are presented as mean ± SD, and categorical variables are presented as absolute numbers and percentages. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired t-test. Categorical variables were analyzed using either the chi-square test or Fisher's exact test. For all statistical tests, a p-value less than

0.05 was taken to indicate a significant difference. Data were checked for normality before statistical analysis. Normally distributed continuous variables are presented as mean ± SD while those with a skewed distribution were described as a median.

RESULTS

Comparison between the dipstick results and the manual count results

A total number of 110 patients with cirrhotic ascites were studied who were presented to the Jaipur Golden Hospital, New Delhi, India with the complaint of abdominal distension. A total of 20 patients were diagnosed with SBP by the manual cell count method which is considered a gold standard for the diagnosis of SBP, and 90 patients were diagnosed as non-SBP. We re-diagnosed these 20 patients with SBP with a dipstick method, the dipstick method showed a positive result for 18 patients (90%) and a negative result for 2 patients (10%). In the SBP group, the dipstick showed 18 true positives and 2 false negatives. In the non-SBP group of 90 patients, the dipstick method showed negative results in all the patients with no positive results. Thus, the dipstick showed 100% true negatives with 0% false positives. The prevalence of SBP in patients with cirrhotic ascites in a hospital was found at 18.2%.

Clinical presentation

In 20 patients in the SBP group, 13 patients (65%) had a fever whereas 7 patients were afebrile (35%) while in 90 patients in a non-SBP group, 46 patients (51.1%) had a fever, and 44 patients (48.9%) were afebrile. 70% of the patients in the SBP group had abdominal pain and 30% of patients had no abdominal pain while in the non-SBP group only 31.1% of the patients had abdominal pain while 68.9% of patients had no abdominal pain. 17 out of 20 patients with SBP had jaundice (85%) whereas 45 patients out of 90 in the non-SBP group had jaundice (50%). The SBP group had a mean value of total leucocyte count (TLC) of 19304 whereas the non-SBP group had a mean value of TLC of 10487. 90% of the patients had either straw color or yellow fluid in the SBP group whereas 95% had a similar fluid in the non-SBP group (not significant). Both the patients with hemorrhagic ascites had SBP (p-value 0.032). None of the patients with SBP had transparent ascitic fluid. The median value of the manually counted ascitic fluid PMNs in the patients with SBP was 1453 and the median value of PMNs in the non-SBP group was 56. The difference was statistically significant with a p-value of <0.001. Similarly, the median value of ascitic fluid protein and ascitic fluid albumin in the patients with SBP were 1.65 and 1.40 respectively and the corresponding values in the non-SBP were 1.9 and 1.25 respectively. The statistical difference in the ascitic fluid protein between the two groups was significant with a p-value of 0.020.

DISCUSSION

By using 250 as the criteria for the diagnosis of SBP, we got the sensitivity of dipstick 90%, specificity of 100%, positive predictive value of 100%, negative predictive value of 97.8%, and accuracy of 98.2%. 90% of the patients that were documented as having SBP by the manual cell count, showed the positive result with the dipstick and 10 % of the patients with SBP showed false-negative results. In the false negatives, PMN cell count was low in the range of 270 to 320. In this instance perhaps the PMN maybe not be activated, thereby not releasing the leucocyte esterase.[17] This may explain to some extent the observed negative test result. Moreover, antibiotics can produce both false-positive and false-negative results.[18] The overall result was true positives 18 out of 20 positives with SBP, true negatives 90 out of 90 patients without SBP, false negatives 2, and false positives nil. In addition, the prevalence of SBP in patients with cirrhotic ascites in our hospital was also evaluated which was our secondary objective, and was 18.2%.

Different types of dipsticks have been used in different places e.g combur 2 LN, multistix 10 S, multistix 8SG, Auction, and nephur stick which have got different sensitivities and predictive values for the

diagnosis of SBP in cirrhotic ascites. In most of the dipsticks, the PMN cell count is shown by color change. The dipstick which we evaluated is easily available and can be used with a simple urometer to provide the cell count. So, keeping the cost value and availability also in mind in addition to the rapid diagnosis of SBP, we used the SD Urocolor 10 dipstick (standard diagnostics) for the rapid screening of SBP in cirrhotic patients with ascites. Each dipstick costs only 15 rupees and makes it affordable. As the screening test should be highly sensitive and cheap, we found this dipstick superior to all other dipsticks used earlier in different studies. Thus, the use of this diagnostic modality could save lives by prompting early therapy.

CONCLUSION

The leukocyte esterase dipstick test for ascitic fluid has a high sensitivity, specificity, and positive predictive value for the rapid diagnosis of SBP and may be used for the rapid bedside diagnosis of SBP especially when the delay in the manual count is expected. The prevalence of SBP in cirrhotic ascites was 18.2%.

Conflict of Interest
The author declares that there are no conflicts of interest relevant to this article.

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