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Positive Leukocyte Esterase Dipstick Test: Association With Spontaneous Bacterial Peritonitis In Patients Suffering From Liver Cirrhosis

Sumbul Shahzadi[1], Fuad Ahmad Siddiqi[2], Imran Khan[3], Kanaz Ahmad Siddiqi[4], Javed Ahmad Khan[5], Ayesha Ashraf Cheema[6]

Abstract

Objective: To assess the diagnosing utility of the leukocyte esterase dipstick test by determining the association of a positive leukocyte esterase dipstick test with spontaneous bacterial peritonitis (SBP) in patients suffering from liver cirrhosis.

Methods: This was a cross-sectional study conducted at the Department of Medicine, Combined Military Hospital (CMH), Rawalpindi from January 2023 to December 2023. This study included a total of 65 patients with liver cirrhosis with ascites that fulfilled the exclusion and inclusion criteria. 50 mL of ascitic fluid was drawn from every patient. Afterwards, a dipstick test strip (URS-10A strip) was carefully introduced into each sample. The colour that appeared on the dipstick after 2 minutes was observed and that colour was compared with the colour significance chart mentioned on the dipstick box. Meanwhile, 100ml of the same sample of ascitic fluid was sent to a laboratory for routine evaluation of leucocyte count for the confirmation of diagnosis for SBP. The test results of the two investigations were compared using a Chi-square test. A p-value less than 0.05 was considered to be statistically significant.

Results: A total of 65 individuals participated in our study. Out of the total, 39 (60.0%) were male with the mean age of 57.03±9.063, and 26 (40.0%) were females with the mean age of 55.54±8.453. A Chi-square was performed to assess the association between the dipstick test & ascitic fluid routine evaluation results. The Dipstick test was found to have 85.18% sensitivity and 92.11% specificity ($p<0.0001$).

Conclusions: In conclusion, it can be stated that although various studies have reported variations in the positive predictive values (PPV) and sensitivity value, the accurate calculation of negative predictive value (NPV) in the majority of studies affirms the beneficial use of leukocyte esterase test strips in healthcare settings for patients suffering from cirrhosis-related asities with spontaneous bacterial peritonitis.

MeSH Keywords: Ascites, Leukocyte, Liver cirrhosis, Peritonitis.

[1,6] Registrar Medicine, CMH, Rawalpindi; [2] Professor of Medicine, CMH, Rawalpindi; [3,5] Assistant Professor of Medicine, CMH, Rawalpindi; [4] Medical Officer, CMH, Rawalpindi.

Correspondence: Dr. Sumbul Shahzadi, Registrar Medicine, CMH, Rawalpindi. **Email:** sumbulshahzadi619@yahoo.com

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1. Introduction

Spontaneous bacterial peritonitis (SBP) is a serious complication that can occur in individuals with cirrhosis, alongside variceal bleeding. It is crucial to promptly diagnose and treat SBP to improve the chances of patient survival.[1] Among cirrhotic patients, SBP is one of the most frequent and severe complications, accounting for 10-25% of cases. It can further lead to multi-organ failure, thus affecting the patient's prognosis. So it is crucial to promptly diagnose and treat it to improve the chances of patient survival.[2] Initially, the mortality rate for SBP was over 90% when reported, but due to improvised diagnostic modalities and more understanding of the pathophysiology of the disease, patients are now diagnosed earlier and the mortality rate has decreased to around 20%.[3] SBP is characterized by an infection spontaneously developing in the ascitic fluid without an intra-abdominal source of infection. Patients with SBP typically experience symptoms such as abdominal pain, fever, hypotension, impaired renal function, and potential progression to encephalopathy.[4] The clinical signs of SBP can be subtle and gradual, necessitating a high level of clinical suspicion for early diagnosis. Additionally, many SBP patients may exhibit no symptoms at all, in such situations clinical diagnosis without a paracentesis can be ambiguous. In the current era, the complications of SBP have decreased by approximately 30%-40% due to early detection and treatment involving appropriate management.[5] Despite significant advancements in medical care for individuals with advanced liver disease, SBP remains a consequential problem and a leading cause of bacterial infection-related mortality in these patients.

Although the exact cause of spontaneous bacterial peritonitis is unknown, the most widely accepted theory recommends the following series of events: I) intestinal bacteria translocation through the gut mucosa into mesenteric lymph vessels; II)

contamination of the bloodstream by bacteria of infected lymph; III) an extended period of bacterial presence in the blood due to the compromised activity of phagocytes in the reticuloendothelial system; IV) the introduction of bacteria from the bloodstream into the ascitic fluid; and V) the unrestricted proliferation of bacteria in ascites that lacks adequate levels of opsonins.[6]

Presently the gold standard test to detect spontaneous bacterial peritonitis (SBP) rely on identifying polymorphonuclear (PMN) cells by counting chamber method in the fluid surrounding the abdominal organs. This technique is based on the theory that when PMN cells reach approximately 250 per cubic millimetre in the ascitic fluid, it indicates the presence of peritonitis.[7] However, using a cell counter method to diagnose SBP can be time-consuming, taking several hours. In addition, the lack of sufficient and necessary laboratory facilities, as well as the prolonged duration of ascitic fluid cultures, contributes to delays in diagnosing SBP through this method. Thus, an urgent need for a quick diagnostic test arises. Enter the leukocyte esterase reagent (LER) dipstick, a test that eliminates the time constraint by enabling the diagnosis of peritonitis within a matter of minutes.[8]

Lately, the examination of leukocyte esterase activity through dipstick testing has become a popular method for swiftly identifying infections in ascitic fluid.[9] The esterase released by polymorphonuclear (PMN) cells reacts with an esterified chemical compound present in the test strip, resulting in the formation of a violet azo dye. The intensity of this dye is directly related to the quantity of leukocytes present.[10]

Numerous recent studies have demonstrated the dipstick's effectiveness in diagnosing spontaneous bacterial peritonitis (SBP). However, it is important to note that the strip tests employed in these studies differ from one another and employ various colourimetric scales. Consequently, the sensitivity and accuracy of these different dipsticks may vary. Thus far, there has been no universally recommended colour scale for each dipstick when diagnosing SBP.

In Pakistan, only a few studies have been conducted to determine the association of spontaneous bacterial peritonitis in patients with liver cirrhosis using positive leukocyte esterase dipstick test. This study is designed to know the significance and diagnosing utility of this simple and economical test in our resource restraint population.

2. Materials & Methods

This cross-sectional study was performed at the Department of Medicine, Combined Military Hospital (CMH), Rawalpindi from January 2023 to December 2023 after obtaining approval from the Institutional Review Board (IRB), vide reference number 528. Sampling was done using a non-probability consecutive sampling technique. Patients of liver cirrhosis with ascites visiting the medical OPD or admitted to the medical ward with a history of fever, pain abdomen, or abdominal tenderness during our research study duration were included in this study. Patients having a history of abdominal surgery in the last 4 weeks prior, patients who had been kept on antibiotics for more than 12 hours at the time of recruitment, and those with a diagnosis of peritoneal malignancy were excluded from this study.

Written consent for inclusion in the study from all the included patients was taken. The advised treatment to the patients proceeded as planned without any modifications or delay. Apart from the routine sampling based on each patient's needs, we collected a 50 mL sample of ascites for the LER dipstick test. The samples underwent interpretation according to the LER dipstick manufacturer's guidelines. Additionally, we sent the samples to the laboratory of our hospital for ascetic fluid routine evaluation and standard test to determine cell count by counting chamber method for confirmation of SBP.

The leukocyte esterase dipstick test was conducted by pouring the sample of ascites into a test tube. Next, a dipstick test strip (URS-10A strip) was gently placed in the liquid, adhering to the manufacturer's guidelines, for a duration of 1 to 2 seconds. The strip was then placed on a clean surface. After waiting for 2 minutes, the resulting colour was compared to the standard sample and observed. The terms "trace," "small," "moderate," and "large" (alternatively "trace," "1+," "2+," and "3+") were utilized to report the findings.

A typical test strip can contain up to 10 varying pads or substances that undergo a colour change when placed in and subsequently taken out of a sample. This analysis involves checking for the presence of proteins, glucose, ketones, haemoglobin, bilirubin, urobilinogen, acetone, nitrite, and leucocytes. It also entails testing the specific gravity and pH level or detecting infections caused by different pathogens. The gold standard method for diagnosing SBP considers WBC values greater than 250 cells per cubic millimetre or PMN values exceeding 250 cells per cubic millimetre as positive results.

Data analysis was done on SPSS version 25.0. Quantitative variables like age were measured as mean ± SD. Qualitative variables like gender distribution and dipstick test results were measured as frequency and percentages. The chi-square test was used to calculate the association between dipstick results and findings of ascitic fluid cell count by counting chamber method; $p<0.05$ was considered significant. The sensitivity and specificity of the dipstick test were calculated.

3. Results

A total of 65 individuals participated in our study. Out of the total, 39 (60.0%) were male with the mean age of 57.03±9.063, and 26 (40.0%) were females with the mean age of 55.54±8.453. All the characteristics of patients including gender distribution, prevalence of spontaneous bacterial peritonitis, severity based on Child PUGH scores, common etiologies of cirrhosis, and results of a dipstick test in the studied population are shown in Table 1.

Table 1: Demographic and Clinical Characteristics of Cirrhotic Patients

Characteristics		n (%)
Gender	Male	39 (60.0%)
	Female	26 (40.0%)
Spontaneous Bacterial Peritonitis	Positive	27 (41.5%)
	Negative	38 (58.5%)
Child PUGH Score	Class A	11 (16.9%)
	Class B	19 (29.2%)
	Class C	35 (53.8%)
Aetiology of cirrhosis	Hepatitis B	37 (56.9%)
	Hepatitis C	20 (30.8%)
	Cryptogenic Liver Cirrhosis	8 (12.3%)
Dipstick test	Positive	26 (40.0%)
	Negative	39 (60.0%)

The association between Dipstick Test Results and Spontaneous Bacteria Peritonitis (diagnosed via ascetic R/E) was established (Table 2).

Table 2: Association between Dipstick Test Results and Spontaneous Bacterial Peritonitis (SBP)

Dipstick	Spontaneous Bacterial Peritonitis		p-Value
	Positive	**Negative**	
Positive	23 (TP)	3 (FP)	<0.0001
Negative	4 (FN)	35 (TN)	

Sensitivity= TP/(TP+FN)= 23/(23+4)*100= 85.18%
Specificity= TN/(TN+FP)= 35/(35+3)*100=92.11%
Positive Predictive Value= TP/(TP+FP)*100= 23/(23+3)= 88.46%
Negative Predictive Value= TN/(TN+FN)*100=35/(35+4)= 89.74%
Diagnostic Accuracy=(TP+TN)/All patients*100 = (23+35)/65=89.2%

A Chi-square was performed to assess the significance of the relationship between Dipstick Test Results and Spontaneous Bacterial Peritonitis. Dipstick Test had 85.18% sensitivity and 92.11% specificity, p<0.0001. There were significant associations between different levels of Ascitic Fluid Cell count and the Child PUGH Score was found. The strong correlations, particularly the significant p-value (<0.0001), suggested that Ascitic Fluid levels were closely linked to Child PUGH Score in the study population (Table 3).

An Association between Dipstick Test Grades was established, and a significant p-value suggested that there was a significant association between them (Table 4).

Table 3: Association between Ascitic Fluid Cell Count and Child-Pugh Score in Cirrhotic Patients

Ascitic Fluid	Child PUGH Score			Total	p-Value
	Class A	**Class B**	**Class C**		
<250 cells/mm^3	11 (28.9%)	14 (36.8%)	13 (34.2%)	38 (58.5%)	<0.0001
>250 cells/mm^3	0	5 (18.5%)	22 (81.5%)	27 (41.5%)	
Total	11 (16.9%)	19 (29.2%)	35 (53.9%)	65 (100%)	

Table 4: Association between Dipstick Test Grades and Ascitic Fluid R/E Stratified by Cell Count

Dipstick Test	Ascitic Fluid R/E		Total	P-Value
	<250 cells/mm^3	**>250 cells/mm^3**		
Grade 1	30 (93.75%)	2 (6.25%)	32 (49.2%)	
Grade 2	5 (71.4%)	2 (28.6%)	7 (10.8%)	
Grade 3	2 (16.7%)	10 (83.3%)	12 (18.5%)	<0.0001
Grade 4	1 (7.1%)	13 (92.9%)	14 (21.5%)	
Total	38 (58.5%)	27 (41.5%)	65 (100%)	

4. Discussion

Cirrhosis often leads to bacterial infections, which can be a significant problem.[11] These infections are responsible for around 25% to 46% of hospitalizations resulting from acute decompensation in cirrhosis patients.[12] They have high rates of illness and death associated with them. Bacterial infections make the likelihood of death in decompensated cirrhosis patients four times higher. The mortality rate reaches 30% within the first month and 63% within the first year of follow-up.[12]

Spontaneous bacterial peritonitis is the most common bacterial infection found in individuals with cirrhosis of the liver. It is followed by pneumonia, urinary tract

infections, infections of the soft tissues and skin, and spontaneous bacteremia.[13] The primary bacteria responsible for causing this complication, as identified in ascites fluid, include Escherichia coli (approximately 70%), Pseudomonas aeruginosa (approximately 2%), Enterococcus faecalis and Proteus mirabilis (each around 4%), Klebsiella (around 10%), and other unidentified agents (approximately 6%).[14] These infections create endotoxemia, which triggers the release of proinflammatory cytokines such as TNF-α, IL-6, and IL-1. Consequently, this leads to the activation of polymorphonuclear cells (PMN).[15] Recognizing the urgency to avoid the high rates of morbidity and mortality associated with delayed treatment in cirrhotic patients with spontaneous bacterial peritonitis, prompt diagnostic tests are crucial for early detection. Currently, the ascitic fluid PMN count serves as the standard means of determining the appropriate course of treatment for spontaneous bacterial peritonitis.[16]

In this study, we assess the usefulness of leukocyte esterase test strip reagents in quickly diagnosing SBP. Our findings align closely with similar research conducted globally. Our study results found 85.18% sensitivity, 92.11% specificity, 88.46% PPV, 89.74% NPV and 89.2% accuracy using the URS-10A strip. Sarwar *et al*.[17] conducted a similar study and found 97.7% sensitivity, 89.4% specificity, 97.7% PPV, 97.7% NPV and 96.2% accuracy combo 10 test strips. As compared to this, our study revealed lower PPV and sensitivity and higher specificity. Another study by DY and colleagues[18] determined 50% - 67% sensitivity, 100% specificity, 100% PPV and 88% NVP using the Multistix test strip (10SG) and the UriScan test strip.

The variances we noticed in our research, as well as in the referenced studies, can be ascribed to two main factors. Firstly, the variations arise from the different types of test strips used in these studies, leading to a slight variation in results. Secondly, there are variances in the threshold for determining a positive outcome based on the rate of colour change. When studies consider fewer colour changes (lower grades than the test strip) as positive results, the sensitivity is higher while the specificity is comparatively lower.

The leukocyte esterase test was initially designed for urine analysis.[19] However, it has now proven to be beneficial in detecting infections in various body fluids. Several centres have confirmed its effectiveness in identifying infections in ascitic fluid.[20, 21] Nevertheless, previous studies have shown that the commercial dipsticks used in these tests had varying colourimetric scales for measuring the number of PMN cells. Furthermore, there is currently no standardized cut-off colourimetric scale for each type of dipstick.[22]

Our research has certain limitations due to its focus on a single centre and its small sample size. To comprehensively evaluate the pros and cons of leukocyte esterase test strips in diagnosing SBP, it is recommended that additional studies be conducted using a larger sample size.

5. Conclusion

In conclusion, the leukocyte esterase dipstick test proves to be a valuable tool in the rapid diagnosis of spontaneous bacterial peritonitis (SBP) in patients with liver cirrhosis-related ascites. Our study demonstrated the high sensitivity (85.18%), specificity (92.11%), positive predictive value (88.46%), and negative predictive value (89.74%) of the dipstick test. These findings suggest that the leukocyte esterase dipstick test is both accurate and reliable. Furthermore, its ease of use and cost-effectiveness make it a practical option for healthcare settings, especially where resources are limited. Despite various studies have reported variations in the positive predictive values (PPV) and sensitivity value, the accurate calculation of negative predictive value (NPV) in the majority of studies affirms the beneficial use of leukocyte esterase urine test strips in healthcare settings for patients suffering from cirrhosis-related ascites with spontaneous bacterial peritonitis (SBP).

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S.S, I.K, K.A.S - Conception of study
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S.S, I.K, A.S.C - Manuscript Writing
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