Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/14711
DOI URL: http://dx.doi.org/10.21474/IJAR01/14711

RESEARCH ARTICLE

EFFICACY OF ASCITIC ACID DIPSTICK LEUKOCYTE ESTERASE ACTIVITY IN EARLY DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS

Dr. Sanket Mahajan

Manuscript Info

Manuscript History
Received: 10 March 2022
Final Accepted: 14 April 2022
Published: May 2022

Key words:-
Leukocyte Esterase, PMNL, Culture, Spontaneous Bacterial Peritonitis, Liver Cirrhosis

Abstract

Background: Spontaneous bacterial peritonitis is serious medical complication associated with advanced stage of hepatic cirrhosis and requires quick diagnosis for initiating appropriate antibiotics. 'Leukocyte esterase' reagent dipstick works upon 'esterase' activity of activated granulocytes. This enzyme functions by undergoing reaction with an ester-releasing 'hydroxyphenylpyrrole' which causes color changes in azo dye coated reagent strip.
Aim: The aim of present study was to assess the diagnostic importance of leukocyte esterase reagent dipstick in ascitic fluid for diagnosis of SBP.
Materials and Methods: Ascitic fluid sample were obtained from 100 hospitalized patients with liver cirrhosis. PMNL cell count was performed and results were compared with Leukocyte Esterase Dipstick strip test. The strips were observed in 90 seconds by comparing with colorimetric grade scale (from 0 to 4).
Results: 99.1% specificity, 100% sensitivity, 93% PPV and 100% NPV were obtained for diagnosing SBP.
Conclusion: Leukocytic esterase reagent (dipstick test) is a specific, quick, less expensive as well as simple bed-side method for arriving at diagnosis of spontaneous bacterial peritonitis. Negative strip test can be used for excluding a SBP diagnosis.

Introduction:-

Poly-morphonuclear lymphocyte (PMNL) cell counting in ascitic fluid is important for diagnosing and managing spontaneous bacterial peritonitis.[1] Spontaneous bacterial peritonitis is a common as well as severe type of complication seen with decompensated type of cirrhosis. When it was first described in 1970s, mortality rate seen was exceeding 80% and at present, significant improvement in prognosis of spontaneous bacterial peritonitis has been noted. Currently, the mortality rate is around 20%. [2, 3]

Approximately 10 to 30% of cirrhotic patients who are hospitalized report with spontaneous bacterial peritonitis. [4, 5, 6, 7] On the other hand, only 3.5% of patients with cirrhosis are reported in outpatients. [8]

There are a total of 4 practice guidelines as well as expert consensus reports on diagnosis as well as management of Spontaneous Bacterial Peritonitis. According to each of these documents, diagnosis of spontaneous bacterial peritonitis must be completely based upon polymorphonuclear leukocytic cell counts in ascitic fluid. [9, 10, 11]

Corresponding Author:- Dr. Sanket Mahajan

PMNL counts higher than 250 cells/cubic mm is highly suggestive of Spontaneous Bacterial Peritonitis. It acts as an indicator for initiating antibiotic therapy. Just a fraction of Spontaneous Bacterial Peritonitis patients manifest with symptoms that are typically suggestive of peritoneal infectivity for example, pyrexia, abdominal pain and an elevated blood leukocyte count. SBP may be suspected if a patient shows symptoms of hepatic encephalopathy or rapidly progressing impairment in renal functions with no precipitating factor. Additionally, significant percentages of patients, SBP can be entirely asymptomatic. Here, diagnosis can be done by utilizing paracentesis.[12]

After obtaining diagnosis, collection of ascitic fluid along with blood culture must be done prior to beginning of antibiotics administration. Commonest microorganism isolated from patients suffering from SBP is- Escherichia coli, Gram-positive cocci (Streptococcus species, mainly) and Enterococci.

These microrganisms constitute approximately 70% of all SBP cases. [13, 14] However, antibiotics therapy must not be delayed till microbiological culture reports are made available. Also, patients in which conventional bacterial culture is done, an ascitic fluid culture may turn out to be negative in approximately 60% patients diagnosed from SBP. [9] Hence, antibiotics must be started immediately after diagnosis of SBP with paracentesis. The antibiotic of choice is Cefotaxime for treating Spontaneous Bacterial Peritonitis as it is effective upon 95% of microflora found in ascetic fluid and also, attains high concentration in ascetic fluid. [15, 16, 17, 18]

The recommended dosage of cefotaxime is 4 to 8 grams per day through intravenous route for minimum five days. [19]

Urinary reagent strips that can identify leukocytic population by means of detection of ‘esterase’ activity through colorimetric based reaction has been proposed for bed-side diagnosis of Spontaneous Bacterial Peritonitis. These diagnostic strips have been tested for quick diagnosis of bacterial origin meningitis, pleuritis or synovitis.

First study that was published in 2002 comprised of a total of 72 cirrhosis patients of which 9 had Spontaneous Bacterial Peritonitis reported 100% sensitivity as well as specificity of these urine strips for diagnosing SBP. [20]

However, there is conflicting evidence regarding use of diagnostic urinary strips for diagnosis of SBP. Hence, this study was designed to test the efficacy of ascitic acid dipstick leukocyte esterase activity in early diagnosis of spontaneous bacterial peritonitis.

Materials and Methods:-

This prospective analytical study was conducted in Department of Medicine of Dr. Kiran C. Patel Medical College and Research Institute, Bharuch for a period of January 2021 and December 2021 following approval from appropriate institutional ethical committee of our scientific and ethical committees. Written informed consent was obtained from study participants prior to their recruitment in this study.

The study sample comprised of one hundred (100) patients suffering from ascites as a result of decompensated hepatic cirrhosis with varied etiologies. Inclusion criteria for Spontaneous Bacterial Peritonitis patients were diagnosed by symptoms of- fever, generalized pain in abdomen, tenderness, $\geq$ 250 cells/ mm3) PMNLs in ascitic fluid and minimum of one microrganism that has been isolated from culture.

Exclusion criteria for the study were- Hemorrhage associated ascites, secondary type of peritonitis, immunocompromised patients (for example- pregnant females, patients undergoing chemotherapy, suffering from HIV) patients who received antibiotic therapy 10 days before hospitalization, patients with cardiac failure/ ischemic heart disease, type II diabetes, hypertension, hyper-lipidemia hematological disorders (for example, leukemia, myeloproliferative disorders, aplastic anemia, neoplasms) hypo- or hyperthyroidism, auto-immune diseases, those on anticoagulants, Non-Steroidal Anti-Inflammatory Diseases or oral contraceptive drugs 10 days before hospitalization.

Selected patients had been subjected to complete medical history, thorough general physical examination, laboratory tests, i.e., Complete blood count which included- Mean corpuscular volume (M.C.V.), pro-thrombin time (PT), liver functions tests which comprised of total plasma proteins, serum albumin, ALT, AST, total direct serum bilirubin and alkaline phosphatase; renal function tests (for analyzing serum creatinine, urea, blood urea nitrogen), electrolytes (for example, K, Na and Ca), serum alpha-feto protein (AFP) and analysis of ascitic fluid (white blood cells count

/mm3 with absolute neutrophil count/millimeter cube, total protein/albumin, microbiological culture with sensitivity, cytological examination and leukocyte esterase in ascitic fluid by leukocyte esterase reagent strips. All patients were subjected to ultrasound examination of pelvic and abdominal region.

Confirmatory diagnosis of Spontaneous Bacterial Peritonitis was made based on minimum 250 cells per cubic millimeter of Polymorphonuclear leukocytes (PMNLs) in Ascitic fluid along with positive ascitic fluid culture.

Specimen Sampling:-

a. Collection of blood sample: 5 ml of blood was with-drawn by means of venipuncture. Out of the total blood sample collected, 1ml mixed with sodium citrate was stored in a test tube for complete blood counting while 4 ml of blood sample was allowed clotting in an Eppendorf tube. Non-hemolyzed sera was separated. This serum sample was used for determining creatinine, uric acid, C-reactive protein and liver function tests.
b. Collection of ascitic fluid: A light yellow colored ascitic fluid sample was collected by paracentensis under aseptic environment. The collected fluid sample was immediately tested using Dipstick for leukocyte esterase activity. The strip was kept immersed in the fluid sample for 90 seconds after which it was taken out and change in color was compared with standard color chart provided.

The test was considered as 'positive' on appearance of purple color.

Table 1:- Table showing grade of esterase activity and change in color of strip.

Grade	Color	No. of cells per cubic mm
0	Light yellow	Nil
1	Light pink	15 PMNLs/cubic mm
2	Pink	>70 PMNLs/cubic mm
3	Light purple	>125 PMNLs/cubic mm
4	Purple	>500 PMNLs/cubic mm

Statistical analysis was performed after coding the collected observational data using the SPSS statistical software version 10.0 (SPSS Inc. Chicago. 1L, USA). Quantitative data were calculated as arithmetic mean and standard deviation (means ± SD). Sensitivity, specificity, positive predictive value and negative predictive values were calculated.

Results:-

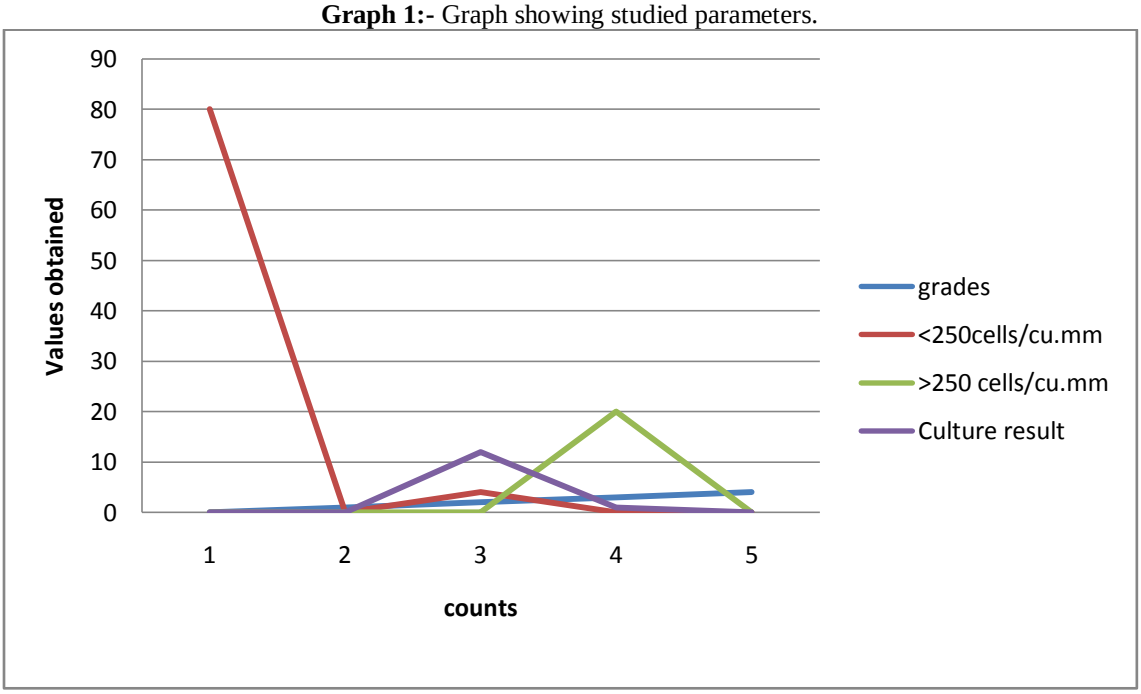
Ascitic fluid was obtained from a total of 100 paracentensis performed on patients suffering from liver cirrhosis. Of the studied sample, spontaneous bacterial peritonitis was diagnosed in 20 patients by means of cytological examination (>250 PMNLs/cubic mm), of these only 12 specimens demonstrated positive microbial culture. All positive patients tested positive with the Dipstick urine strips (grades 2 to 3). The paracentensis procedure was repeated in 5 patients on antibiotic therapy after 48 hours. Of these, in 4 cases the neutrophil count was less than 250 cells/cubic mm, however, the strip test was positive. All patients were then put on revised antibiotics therapy. Although, 1 patient who was undergoing antibiotic therapy had negative strip test as well as cell count less than 250 cells/cubic mm.

In remaining 80 cases, negative colorimetric strip test was found to correlate with PMNL count less than 250 per cubic mm. The cut off threshold value of 250 cells per cubic mm showed 100% sensitivity, 99.1% specificity, 93% positive predictive value and 100% negative predictive value.

Table 1:- Spontaneous bacterial peritonitis cases diagnosed by cytology and Dipstick strip test.

Grades / PMNL count /cubic mm	0	1	2	3	4
<250 cells/cubic mm	80	-	4	-	-
>250 cells/cubic mm	-	-	-	20	-
Cultures	-	-	12	01	-

Graph 1:- Graph showing studied parameters.



Discussion:-

Spontaneous bacterial peritonitis is a serious medical condition which requires fast diagnosis for starting antibiotic therapy. Traditionally, diagnosis of SBP is based upon manual laboratory examination of ascitic fluid which requires long time reporting. This condition is diagnosed from ascetic fluid if PMNL cell count is ≥250 cells per cubic mm.

Hence, there is a pressing need for a newer diagnostic tool that can facilitate quick diagnosis and treatment of patients as it is a fatal condition.

Results of this prospective analytical study showed high accuracy of urinary reagent strips for performing diagnosis of SBP from ascitic fluid sample. The grades 2 and/or 3 test result of reagent test strips demonstrated high positive predictive value (93%) along with 99.1% specificity for diagnosing SBP. Also, in patients with test reagent strip with grades 0 or 1, SBP diagnosis can be excluded as 100% negative predictive value was obtained.

Our study findings are supported by other investigators as well. In conformance with our study, Riggio et al (2009) have reported 85 % to 100% sensitivity and 90% to 100% specificity of urinary strips for diagnosing SBP. [1] Similarly, Braga et al (2006) reported 100% sensitivity, 98.9% specificity, 92.3% Positive predictive value and 100% negative predictive value for urinary reagent strips (Combur test). [20]

In a similar study, Théovenot et al (2004) observed 89% sensitivity and 100% specificity of urine strips (Combur-2).[21] Also, Castellote et al (2003) have demonstrated that urine screening test using Aution sticks showed 96% sensitivity and 89% specificity for detection of SBP in ascites. [22] Vanbiervliet et al (2002) in their study observed that rapid urine screening test using 'Multistix 8SG' demonstrated 100% sensitivity as well as specificity for SBP. [23]

In current study, only 13 (0.13%) positive microbiological cultures were reported. Most common microorganism isolated was Escherichia coli. Similar low percentages have been reported by other studies as well and have ranged between 39% to 59%. Presence of positive microbial culture might be reflective of an early diagnosis of bacterial peritonitis. [23, 24, 25]

Conclusion:-

Urinary test strips for screening and diagnosis of spontaneous bacterial peritonitis is an excellent tool specially, when used as an adjunct with microbiological culture methods. Our study along with similar other studies has demonstrated its high sensitivity and specificity to be used as an effective bed-side diagnostic tool.

Thus, a combination of bed-side strip tests of ascitic fluid coupled with bed-side inoculation in blood based culture broths may decrease numbers of samples of ascetic fluid as 'false negative'. As a result of this, early and specified antibiotics may be prescribed to patients.

References:-

1. Riggio O, Angeloni S. Ascitic fluid analysis for diagnosis and monitoring of spontaneous bacterial peritonitis. World J Gastroenterol 2009;15(31):3845-50.
2. Llovet JM, Planas R, Morillas R, Quer JC, Cabre E, Boix J, Humbert P, Guilera M, Domenech E, Bertran X. Short-term prognosis of cirrhotics with spontaneous bacterial peritonitis: multivariate study. Am J Gastroenterol 1993; 88: 388-392
3. Toledo C, Salmeron JM, Rimola A, Navasa M, Arroyo V, Llach J, Gines A, Gines P, Rodes J. Spontaneous bacterial peritonitis in cirrhosis: predictive factors of infection resolution and survival in patients treated with cefotaxime. Hepatol 1993; **17**: 251-7.
4. Caly WR, Strauss E. A prospective study of bacterial infections in patients with cirrhosis. J Hepatol 1993; **18**: 353-358
5. Pinzello G, Simonetti RG, Craxi A, Di Piazza S, Spano C, Pagliaro L. Spontaneous bacterial peritonitis: a prospective investigation in predominantly nonalcoholic cirrhotic patients. Hepatol 1983; **3**: 545-9.
6. Almdal TP, Skinhoj P. Spontaneous bacterial peritonitis in cirrhosis. Incidence, diagnosis, and prognosis. Scand J Gastroenterol 1987; **22**: 295-300
7. Llach J, Rimola A, Navasa M, Gines P, Salmeron JM, Gines A, Arroyo V, Rodes J. Incidence and predictive factors of first episode of spontaneous bacterial peritonitis in cirrhosis with ascites: relevance of ascitic fluid protein concentration. Hepatol 1992; **16**: 724-7.
8. Gilbert JA, Kamath PS. Spontaneous bacterial peritonitis:an update. Mayo Clin Proc 1995; **70**: 365-70.
9. Rimola A, Garcia-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, Inadomi JM. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document.International Ascites Club. J Hepatol 2000; **32**: 142-153
10. Runyon BA. Management of adult patients with ascites due to cirrhosis. Hepatology 2004; 39: 841-56.
11. Moore KP, Wong F, Gines P, Bernardi M, Ochs A, Salerno F et al. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. Hepatol 2003; 38: 258-66.
12. Pinzello G, Simonetti RG, Craxi A, Di Piazza S, Spano C, Pagliaro L. Spontaneous bacterial peritonitis: a prospective investigation in predominantly nonalcoholic cirrhotic patients. Hepatol 1983; **3**: 545-9.
13. Garcia-Tsao G. Current management of the complications of cirrhosis and portal hypertension: variceal hemorrhage, ascites, and spontaneous bacterial peritonitis. Gastroenterol 2001; **120**: 726-48.
14. Garcia-Tsao G. Spontaneous bacterial peritonitis. Gastroenterol Clin North Am 1992; **21**: 257-275
15. Felisart J, Rimola A, Arroyo V, Perez-Ayuso RM, Quintero E, Gines P, Rodes J. Cefotaxime is more effective than is ampicillin-tobramycin in cirrhotics with severe infections. Hepatol 1985; **5**: 457-62.
16. Runyon BA, McHutchison JG, Antillon MR, Akriviadis EA, Montano AA. Short-course versus long-course antibiotic treatment of spontaneous bacterial peritonitis. A randomized controlled study of 100 patients. Gastroenterology 1991; **100**:1737-42
17. Rimola A, Salmeron JM, Clemente G, Rodrigo L, Obrador A, Miranda ML, Guarner C, Planas R, Sola R, Vargas V. Two different dosages of cefotaxime in the treatment of spontaneous bacterial peritonitis in cirrhosis: results of a prospective, randomized, multicenter study. Hepatol 1995; **21**: 674-9.
18. Runyon BA, Akriviadis EA, Sattler FR, Cohen J. Ascitic fluid and serum cefotaxime and desacetyl cefotaxime levels in patients treated for bacterial peritonitis. Dig Dis Sci 1991;36: 1782-6.
19. Mercader J, Gomez J, Ruiz J, Garre MC, Valdes M. Use of ceftriaxone in the treatment of bacterial infections in cirrhotic patients. Chemother 1989; 35Suppl 2: 23-6.
20. Braga LLBC, Souza MLHP, Barbosa AMC, Fartado FM, Campelo PAM, Filho AHA. Diagnosis of spontaneous bacterial peritonitis in northeastern Brazil by use of rapid-urine-screening test. Sao Paulo Med J. 2006;124(3):141-4.
21. Thevenot T, Cadranel JF, Nguyen-Khac E, et al. Diagnosis of spontaneous bacterial peritonitis in cirrhotic patients by use of two reagent strips. Eur J Gastroenterol Hepatol 2004;16(6):579-83
22. Castellote J, Lopez C, Gornals J et al. Rapid diagnosis of spontaneous bacterial peritonitis by use of reagent strips. Hepatol. 2003;37(4):893-6.
23. Vanbiervliet G, Rakotoarisoa C, Filippi J, Guerin O, Calle G, Hastier P et al. Diagnostic accuracy of a rapid urine-screening test (Multistix8SG) in cirrhotic patients with spontaneous bacterial peritonitis. Eur J Gastroenterol Hepatol 2002; 14:1257-60.

24. Navasa M, Follo A, Llovet JM, et al. Randomized, comparative study of oral ofloxacin versus intravenous cefotaxime in spontaneous bacterial peritonitis. Gastroenterology. 1996;111(4):1011-7.
25. Fernandez J, Navasa M, Gomez J et al. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfl oxacin prophylaxis. Hepatology. 2002;35(1):140-8.
26. Rimola A, Salmeron JM, Clemente G, et al. Two different dosages of cefotaxime in the treatment of spontaneous bacterial peritonitis in cirrhosis: results of a prospective, randomized, multicenter study. Hepatol 1995;21(3):674-9.