



RAPID BEDSIDE DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS BY USING URINARY REAGENT STRIPS

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ABSTRACT

BACKGROUND: Spontaneous Bacterial Peritonitis (SBP) is the common and fatal complication of cirrhotic patient with ascites and is defined as, "presence of WBC count of 500/cubic mm or more or Neutrophil count of 250 /cubic mm or more in ascitic fluid with mono microbial positive culture, in the absence of an intra abdominal sources of infection".

PATIENTS & METHODS: This prospective and observational study was conducted at Medical Ward-1 (Liaquat University of Medical & Health Sciences Jamshoro, Sindh)/Hyderabad from 01st December 2008 to 30th November 2009. An established cases of liver cirrhosis with ascites having signs & symptoms of SBP were included & those proved to be with secondary peritonitis due to appendicitis, intestinal obstruction or perforation or trauma & patients with history of antibiotic therapy during past 10 days, all were excluded from the study.

RESULTS: Two hundred six cases of cirrhosis underwent ascitic fluid analysis, 112 were Male and 94 were Female, with M:F sex ratio 1.2:1. Mean age was between 20-60 Years. SBP was found in 36 (17.5%) patients by counting chamber method (i.e.; PMN > 250 /cubic mm) & SBP was found in 32 (15.5%) patient with 4-grade colorimeter Reagent strip method (i.e.; grade - 3 positive).

Statistical comparative analysis was carried out between PMN count in ascitic fluid by counting chamber and 4-grade scale colorimetric reagent strip & efficacy of reagent strip for the diagnosis of SBP was evaluated by determining Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value & Accuracy as; 88.9%, 98.7%, 76.1%, 96.2% & 91.3% and M : F ratio was 2:1 respectively.

CONCLUSION: Early diagnosis & treatment can decrease mortality and morbidity of SBP, hence rapid bedside testing method by use of Reagent Strips is one of the best & rapid diagnostic tool for SBP at bedside.

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INTRODUCTION:

Spontaneous Bacterial Peritonitis (SBP) is one of the common and fatal complication of cirrhotic patient with ascites and is defined as, "presence of WBC count of 500/ cubic mm or more, or Neutrophil count of 250 / cubic mm or more in ascitic fluid with mono microbial positive culture, in the absence of an apparent intra abdominal sources of infection" Primarily the ascitic fluid in cirrhotic patients is sterile. Approximately 20-30 % of cirrhotic patients with ascites develop SBP however, the incidence is greater than 40 % in the patients with ascitic fluid total protein < 1 G/dl probably due to decreased ascitic fluid opsonic activity.

The exact route of ascitic Micro-organisms have not yet been found, it is believed that translocation of enteric bacteria across the gut wall or mesenteric lymphatics leads to seeding of the ascitic fluid, as may bacteria from other sites.

Virtually all cases of SBP are caused by a mono microbial infection. The most common pathogens are enteric gram negative bacteria (E.coli & Klebsiella pneumoniae) or gram positive bacteria (S. Pneumoniae, S. Viridans & Enterococcus species, whereas Anaerobes never cause SBP. SBP has two following variants:

*Bacterascites (BA).

*Culture Negative Neutrocytic Ascites (CNNA).

Bacterascites (BA): Defined as, "ascitic fluid WBC count > 500/ cubic mm or Neutrophils < 250/ cubic mm with positive mono microbial culture."

Culture Negative Neutrocytic Ascites (CNNA): Defined as, "ascitic fluid Neutrophil count 250 or more/ cubic mm with negative mono microbial culture."

SBP patients usually present with sudden onset of fever or chills, generalized abdominal tenderness or pain, rebound abdominal tenderness & loss of bowel sounds. Predisposing factors & Pathogenesis of SBP:

- Low protein concentration in ascitic fluid (< 1 G /dl).\
- Low serum albumin level.
- Low opsonic factors in ascitic fluid.
- Defective bactericidal activity or chemotaxis of liver Kupffer cells
- Low C 3 & C 4 Complements serum level.
- High serum Bilirubin level.
- Variceal Hemorrhage.
- Marked hyponatraemia (<120 mmol / L).
- Bacteremia etc.

Worldwide the mortality rate of SBP exceeds 30%. However if the disease is rapidly diagnosed and earlier treated, the mortality rate is less than 10%. As the majority of the patients have underlying severe liver disease, therefore, many may die due to liver failure, hepato renal or pulmonary syndrome or bleeding complications from portal hypertension.^{6,7,8,9,10}

The most diagnostic test for SBP is ascitic fluid analysis for cell count & blood culture inoculated at bedside into blood broth bottle but it is costly & time consuming i.e.; needs 48-72 hours for result, therefore an alternate rapid & cheap method for the diagnosis of SBP at bedside, is by using of Urinary Reagent Strips (URS) in the ascitic fluid (i.e.; for result needs only 60-120 sec), hence quick diagnosis & early treatment can save the life of the patients.

PATIENTS AND METHODS:

This prospective and observational study was conducted at Medical Ward-1 (Liaquat University of Medical Sciences) Jamshoro/ Hyderabad from 01st December 2008 to 30th November 2009. An established cases of liver cirrhosis with ascites having signs & symptoms of SBP were included & those proved to be with secondary peritonitis due to appendicitis, intestinal obstruction or perforation or trauma & patients with history of antibiotic therapy during past 10 days, all were excluded from the study. All selected patients were examined clinically & details were recorded in a proforma. All the patients were subjected to the following investigations:

Complete blood count: It was done on an

TABLE : 1.
CO-RELATION BETWEEN REAGENT STRIP & COUNTING CHAMBER RESULTS FOR SBP.

Diagnosis of SBP by Counting Chamber	Diagnosis of SBP by Reagent Strip Grading				Total number of Patients
	0	1	2	3	
SBP Positive	2	0	2	32	36
SBP Negative	150	8	8	4	170
Total	152	8	10	36	206

TABLE (2).
SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE (PPV), NEGATIVE PREDICTIVE VALUE (NPV) AND ACCURACY OF REAGENT STRIP (RS) TESTING METHOD FOR THE DIAGNOSIS OF SBP.

Screening Tests	RS + > 3	Percentage
Sensitivity	$\frac{32}{36} \times 100$	= 88 . 9 %
Specificity	$\frac{150}{152} \times 100$	= 98 . 7 %
P PV	$\frac{32}{(10 + 32)} \times 100$	= 76 . 1 %
N PV	$\frac{152}{(150+8)} \times 100$	= 96 . 2 %
Accuracy	$\frac{188}{206} \times 100$	= 91 . 3%

automated cell counter (Sysmex K-4500).

- Urine DR: It was done by Siemens Multistic (Siemens Health Care Diagnostics Inc. Tarrytown, NY 10591-5097 USA) & Microscopy was done by pathologist.
- X-ray chest: Was done by 100 KV machine (Siemen, Germany) in a standing posture.
- Plain x-ray abdomen: Were also done by 100 KV machine (Siemen, Germany) in sitting (erect) & lying postures. Abdomen ultra sound: Were done on Toshiba u/s machine with sector probe of 3.57 MHZ frequency.
- HBSAg (Acon, Acon Laboratories Inc. San Diego, USA).
- HCV Antibodies (Acon, Acon Laboratories Inc. San Diego, USA).
- Prothrombin Time (Thromboplastin

with Ca++, Bio Menieux Vitek, Inc. Hazelwood, USA).

- LFTs.
- Random Blood Sugar (if required).
- Ascitic fluid Analysis: By use of urine strip reagent stick (Combur 10 Test (Roche Diagnostics GmbH. D-68298 Mannheim, Germany), the ascitic fluid Leukocyte (WBC)Esterase was detected within 60 – 120 seconds, Colorimetric 4- grade scale observation at bedside. A co-relation between Polymorphneutrophils (PMN) & 4- grade scale is suggested by the manufacturer as : Grade 0= 0 PMN / micro L, Grade 1= 25 PMN / micro L, Grade 2= 75 PMN / Micro L & Grade 3= 500 PMN /micro L. In our study Grade 3 was considered positive for SBP.

Simultaneously at bedside, 10 ml of ascitic fluid was immediately inoculated into a commercially available blood broth bottle (Microcystem - 01 Biosanrahx) for bacterial culture & 10 ml was sent to the hospital pathology laboratory for Detail Report & Cytology.

comparative analysis was carried out between PMN count in ascitic fluid by counting chamber and PMN count determined by 4-grade scale colorimetric reagent strip & efficacy of reagent strip for the diagnosis of SBP was evaluated by determining Sensitivity (proportion of patients with positive reagent strip divided by the patients with the SBP diagnosed by the counter chamber), Specificity (proportion of the patients with a negative reagent strip divided by the total number of patients without SBP), Positive Predictive Value (PPV) (proportion of patients with a true positive reagent strip divided by the total number of patients with a positive reagent strip), Negative Predictive Value (NPV) (proportion of true negative reagent strip divided by total number of patients with negative reagent strip) & Accuracy of Reagent Strip was verified by dividing the sum of true positive & true negative by the total number of patients underwent ascitic fluid analysis respectively.

RESULTS

All the selected 206 cases of cirrhosis with ascites underwent ascitic fluid analysis. Out of these 112 were Male and 94 were Female, with mean age between 20 - 60 Years. Male to Female ratio was 1.2:1

Among 206 patients, SBP was found in 36 (17.5%) patients by counting chamber method (i.e.; PMN > 250 / cubic mm) while SBP was found in 32 (15.5%) patient with 4-grade colorimeter Reagent strip method (i.e.; grade – 3 positive).

Among 36 SBP positive patients, 02 were found in grade -2 and other 02 were in grade -0 on Reagent Strip method. In SBP positive patients male to female ratio was 2:1. Whereas 170 patients were SBP negative on counting chamber method & 150 were SBP negative on reagent strip method. While 02 were in grade-0, 08 were in grade-1 & also 08 were in grade-2 respectively shown in Table-1.

The screening (Gold standard) tests; Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) & Accuracy for grade-3 on Reagent Strip have been calculated in Table-2.

DISCUSSION:

Spontaneous Bacterial Peritonitis is very common & morbid complication of cirrhosis

with ascites. Approximately 20-30% of ascitic patients develop SBP, however the incidence is greater than 40%, in the patients having total ascitic protein < 1 Gm/dl due to low opsonic activity of Leukocytes. Therefore to decrease mortality & morbidity its necessary to diagnose SBP earlier & start treatment atonce.

In our study out of 206 ceases of cirrhosis with ascites, SBP was found in 36 (17.5 %) cases on counter chamber method, while SBP was found in 32 (15.5 %) cases on reagent strip method respectively. With reference to reagent strip method the sensitivity, specificity, PPV, NPV & accuracy were orderly found as; 88.9%, 98.7%, 76.1%, 96.2% & 91.3% & Male to Female ratio was 2:1.

Our results match with other studies conducted in various centres either in home country or abroad Vanbiervliet G et al , comparatively found high sensitivity & specificity i.e.; 100% by use of Multistic 8 SG in cirrhotic patients for screening of SBP. Castellote J et al ¹², showed 89% sensitivity, 99% specificity, 98% PPV and 97% NPV for SBP by use of Aution strips. Here our results match with sensitivity, specificity and NPV.

Another study by Thevenot T et al ¹³, who used Combur-2 test LN & found 89% sensitivity, 100% specificity & PPV and 99% NPV respectively. In this study our results match with sensitivity and specificity. Butani RC et al, ¹⁴, by using Multistics 10 SG found 89% sensitivity, 99% specificity, 89% PPV & 99% NPV. In this study our results also match with sensitivity and specificity.

Also Sapey T et al ¹⁵, in their study by using two different reagent strips at two separate centres i.e.; at centre-1 (France) by use of Nephur-Test strip found 86% sensitivity, 100% specificity & PPV and 99% NPV and at same centre by use of Multistics SG 10, they found 100% sensitivity, specificity, PPV & NPV each. Whereas at centre-2 (USA) by use of Nephur-Test strip, they found 100% sensitivity & NPV, 92.5% specificity and 75% PPV. Where as at same centre by use of Multistics SG 10 , they found 83 % sensitivity, 96% specificity, 83% PPV & 96% NPV. Collectively here our results are similar in regard to sensitivity, specificity, PPV and NPV.

Kim DY et al ¹⁶, in their study by using UriScan- Multistics 10 SG, found 67/50 sensitivity, 100/100 specificity & PPV and 89/87 NPV. In this study our results nearly match in all respects.

In an other study Sapey T et al ¹⁷, by use of Nephur test – Multistics 10 SG found

88/65 sensitivity, 100/100 specificity, 94/92 PPV & 99/97 NPV respectively. Comparatively, in this study all parameters are higher than our parameters. Another local study conducted at Shaikh Zayed Postgraduate Medical institute Lahore by Sarwar S et al ¹⁸, by use of Combur 10 reagent strip, found 97.7% sensitivity, 89.4% specificity, 90% PPV, 97.7% NPV and 96.2% accuracy. Here our results match with NPV and Accuracy.

Braga LLBC et al ¹⁹, at Northeastern Brazil found 100% sensitivity , 98.9% specificity, 92.3% PPV and 100% NPV. Here only specificity match with our result. Rerknimitr R et al ¹⁹, by using Combur 10 Test M, Roche Mannheim, Germany found prevalence of SBP 21%, sensitivity 88%, specificity 81%, PPV 55%, NPV 96% & Accuracy 83% . Here our results match with sensitivity and NPV

More recent study conducted by Nousbaum JB et al ²¹, by use of multistic 8 G, found sensitivity 45.3%, specificity 99.2%, PPV 77.9% & NPV 96.9%. Here specificity PPV and NPV match with our results respectively.

CONCLUSION:

As SBP is one of the common & morbid complication of cirrhosis with ascites therefore its rapid diagnosis and earlier treatment is necessary to decrease its mortality and morbidity. In this regard rapid bedside testing method by use of Urinary Reagent Strips is one of the best, cheap & rapid diagnostic tool for SBP at bedside.

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