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Research Article

STUDY OF ASCITIC FLUID FOR DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS (SBP) IN ADULT PATIENTS WITH CIRRHOSIS

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ABSTRACT

Ascitic fluid infections are a frequent complication among patients with cirrhosis and ascites, of which Spontaneous Bacterial Peritonitis (SBP) is the most common, potentially fatal cause of deterioration in patients with advanced cirrhosis with ascites. One hundred consecutive hospitalized patients of cirrhosis with ascites were included in this prospective study. Ascitic fluid was analysed by culture by the conventional method and direct bedside inoculation in tryptic soy broth (TSB), cell count and biochemical parameters. The cases were categorized into three groups – Classic Spontaneous Bacterial Peritonitis (SBP), Culture Negative Neutrocytic ascites (CNNA) and Monomicrobial non-neutrocytic bacterascites (MNB). Statistical analysis was done by Chi Square test, Paired and Unpaired 't' tests. Out of 100 patients of cirrhosis with ascites, 58% were SBP cases and 42 % were non-SBP cases. Amongst the 58 SBP cases, 53.45% cases were of Classic SBP, 36.21 % were CNNA and 10.34% cases were of MNB. Alcoholism (84.48 %) was the commonest etiology in SBP cases. *Escherichia coli* was the commonest bacteria isolated, followed by *Pseudomonas aeruginosa* and *Acinetobacter* species. Mean ascitic fluid polymorphonuclear count (PMN) count was 755.19 ± 788.04 / mm^3. Overall susceptibility of bacteria to aminoglycosides, quinolones and cephalosporins was 92.31%, 79.17% and 58.33% respectively. Hepatic encephalopathy was the major complication (46.55%) in SBP cases. Highest mortality was seen in CNNA (66.67 %), followed by Classic SBP (45.16 %).SBP is a serious complication in patients with advanced cirrhosis. It has high prevalence, high recurrence rate and poor long term prognosis. A diagnostic paracentesis should always be performed routinely within 24 hours of admission and ascitic fluid sample should be sent for culture, cytology and estimation of biochemical parameters, so as to initiate prompt management in these patients and achieve a better survival rate.

INTRODUCTION

Ascites is a common complication of cirrhosis of liver and indicates the presence of portal hypertension and hepatic decompensation.[1] Spontaneous Bacterial Peritonitis (SBP) is the most common, potentially fatal, yet reversible cause of deterioration in patients with advanced cirrhosis with ascites.[2] SBP is defined as the infection of previously sterile ascitic fluid without an apparent intra-abdominal source of infection.[3] Ascitic fluid infection is classified into 5 categories based on ascitic fluid culture, polymorphonuclear (PMN) count and presence or absence of surgical source of infection.[4]

The variants of Spontaneous Bacterial Peritonitis are Classic SBP, Culture Negative Neutrocytic Ascites (CNNA) and Monomicrobial Non-neutrocytic Bacterascites (MNB). Classic Spontaneous Bacterial Peritonitis (SBP) is diagnosed, when there is a positive ascitic fluid culture, elevated ascitic fluid PMN count $\geq$ 250 cells/mm[3] and no evidence of an intra-abdominal surgically treatable source of infection. Culture Negative Neutrocytic Ascites (CNNA) is diagnosed when ascitic fluid culture grows no bacteria, ascitic fluid PMN count is $\geq$ 250 cells/mm^3, no antibiotic have been given (not even a single dose) and no other explanation for an elevated ascitic PMN count (e.g. haemorrhage into ascites, peritoneal carcinomatosis, tuberculosis or pancreatitis) can be identified. Monomicrobial non-neutrocytic bacterascites (MNB) is diagnosed when there is a positive ascitic fluid culture for a single organism, an ascitic fluid PMN count < 250 cells/mm^3 and no evidence of an intra-abdominal surgically treatable source of infection. Secondary bacterial peritonitis is diagnosed when ascitic fluid culture is positive (usually for multiple organisms), PMN count is $\geq$ 250 cells/mm^3 and an intra-abdominal surgically treatable primary source of infection (peritoneal intestine, perinephric abscess) has been identified. Polymicrobial bacterascites is diagnosed when multiple organisms are seen on Gram stain which is cultured from the ascitic fluid and PMN count is < 250 cells/mm.[3]

Prevalence of SBP in cirrhotic patients varies in different parts of the world. In India, it ranges from 9.3% - 34.92%,[5,6] in other Asian countries from 22% - 58%[7,8] and in the Western countries from 7.7% - 35.4%.[9,10] In India, the mortality ranges from 27.2 % - 43%,[6,11] in other Asian countries from 15% - 35%[7,12] and in the Western countries from 15.45% - 50 %.[13,14]

There are reports mentioning the clinical aspects and treatment of SBP, but the microbiological investigations for etiology of SBP are lacking in these reports. There is also scarcity of documented reports on prevalence of SBP in this part of India and in view of high rate of morbidity and mortality associated with SBP, this study was carried out.

MATERIALS AND METHODS

This was a prospective study of one year duration from January to December 2010. One hundred consecutive adult patients admitted in this tertiary care hospital with cirrhosis were studied after clearance from Institutional Ethics Committee. Ascites due to renal, cardiac, tubercular and malignant pathology, secondary peritonitis and HIV positive patients were excluded from the study.

Ten ml of ascitic fluid was tapped from the patient under proper aseptic precautions. Five ml of it was inoculated directly into 25 ml of tryptic soy broth at the bedside.[5] Tryptic soy broth was incubated at 37^0C and 3 subcultures were done on Blood Agar (BA) and MacConkey Agar (MA) after 24 hours (2^{nd} day), 72 hrs (4^{th} day) and on the 7^{th} day. Two ml of the fluid was centrifuged at 1500 rpm for 10 minutes. Supernatant was discarded. Deposit obtained was utilized for Gram staining and then processed by conventional method on BA and MA.[11] BA was incubated at 37^0C at 5% CO_2 atmosphere for 48 hours. MA was incubated at 37^0C for 48 hours. BA was

incubated at 37^0C at 5% CO_2 atmosphere for 48 hours. MA was incubated at 37^0C for 48 hours. Any growth in BA and/or MA plates was identified by standard biochemical tests. For all the isolates, antibiotic susceptibility pattern was done by Kirby Bauer disc diffusion method (KBDDM) on Mueller Hinton Agar (MHA), according to CLSI guidelines.[15] Remaining 3 ml was put in EDTA bulb for cell count and protein estimation.

Statistical analysis was carried out for significance by calculating the 'p' value. The categorical data was analysed by Chi square test and Fischer exact correction test Open Epidemiological Statistics for public health (Open Epi) software version 2.3). The quantitative data was expressed as mean ± SD and analysed by Unpaired 't' test (Student's 't' test).

RESULTS

One hundred patients of hepatic cirrhosis with ascites were studied, of which 93 were male and 7 were female. 59% of cases were in the age group of 41-60 years. Only alcoholism was the etiology in 85% cases, followed by 9% HBsAg reactive, 5% anti-HCV reactive and 1% anti-HEV reactive patients**.**

Out of total 100 cases, number of SBP cases was 58 (58 %). Amongst 58 SBP cases, Classic SBP was the most common variant - 53.45 % (31/58), followed by MNB - 36.21 % (21/58) and CNNA - 10.34% (6/58). Growth by only TSB was seen in 63.46% cases (33/58) and growth by both the methods (Conventional + TSB) was seen in 36.54% cases (19/58). Thus, all culture positive cases of SBP (52) showed growth in tryptic soy broth (TSB) by direct bedside inoculation (100%). Males predominated (89.66%) in SBP cases. Alcoholism was the commonest etiology of SBP (84.48%), followed by 8.62% HBsAg reactive and 6.90% anti-HCV reactive patients.

By Chi square test, growth by direct bedside inoculation in TSB was statistically significant in comparison to conventional method in 100 cases ($p < 0.00001$) as well as in 58 SBP cases ($p < 0.000001$).

By conventional method, 19 bacteria were recovered – 18 (94.74%) Gram negative bacilli and one (05.26%) Gram positive cocci. By direct bedside inoculation in TSB, 52 bacteria were recoverd – 41 (78.85%) Gram negative bacilli and 11 (21.15%) Gram positive cocci. Amongst the Gram negative bacilli, *Escherichia coli* was the commonest bacteria, 11 (57.89%) by conventional method and 21 (40.38%) by inoculation in TSB (Table 1). Whereas *Escherichia coli* was commonest in Classic SBP, *Pseudomonas aeruginosa* was more in MNB. (Table 2).

82 % (82/100) of the patients belonged to Child Turcotte Pugh class C, 17% to class B and only 1% to class A. Fever, pain in abdomen, yellowish discoloration of body and sclera, altered mental status and oliguria were the common clinical features in 68.97%, 82.76%, 82.76%, 53.45% and 25.86% respectively in SBP cases. They were statistically significant in SBP as compared to non-SBP cases by Chi square test.

Total leucocyte count (TLC) was (mean ± SD) 11331.1 ± 7542.15 / mm^3 in SBP cases and 7736.36 ± 4034.05 in non SBP cases. Serum bilirubin was comparatively higher in the SBP cases with a (mean ± SD) 6.12 ± 7.41 mg/dl than the non-SBP cases (4.25 ± 4.3 mg/dl). Serum creatinine was (mean ± SD) 1.73 ± 1.67 mg/dl in SBP cases and 1.18 ± 0.68 in SBP cases. SGOT levels (mean ± SD) 102.14 ± 95.13 U/L in SBP cases and 69.55 ± 40.11 in SBP cases. Serum protein and albumin levels in SBP cases were (mean ± SD) 6.12 ± 1.20 g/dl and 2.33 ± 0.71 g/dl respectively. Total white blood cell (WBC) count, serum creatinine and SGOT levels were statistically significant in SBP cases as compared to non-SBP cases by Unpaired 't' test.

Ascitic fluid total leucocyte count (1044.40 ± 1013.26/ mm^3), polymorphonuclear count (755.19 ± 788.04/ mm^3) and lymphocyte counts (254.02 ± 215.60 / mm^3) were statistically significant in SBP cases as compared to non-SBP cases.

Table 3 shows the biochemical parameters in the three variants of SBP. By Unpaired 't' test, ascitic fluid PMN count was found to be statistically significant between Classic SBP and MNB (p = 0.0000001) and between CNNA and MNB (p = 0.02585). However, it showed no statistical significance between Classic SBP and CNNA (p = 0.4494). By Unpaired 't' test, ascitic fluid protein was not found to be statistically significant between any of the variants - Classic SBP and MNB (p=0.1215), CNNA and MNB (p= 0.1037) and Classic SBP and CNNA (p=0.6010). By Unpaired 't' test, serum bilirubin was found to be statistically significant between CNNA and MNB (p= 0.0354). However, it showed no statistical significance between Classic SBP and MNB (p=0.2113) and between Classic SBP and CNNA (p=0.1183). By Unpaired 't' test, serum creatinine was found to be statistically significant between Classic SBP and MNB (p= 0.02158). It was not found to be statistically significant between CNNA and MNB (p=0.3504) and between Classic SBP and CNNA (p=0.5437).

Overall susceptibility of bacteria to aminoglycosides, quinolones and cephalosporins was 92.31%, 79.17% and 58.33% respectively (Table 4). All Gram negative bacilli were 100% susceptible to amikacin and imipenem, followed by 91.43% to piperacillin-tazobactam, 80% to ciprofloxacin, 54.29% to cefotaxime and only 20.25% to amoxycillin-clavulanic acid. *Escherichia coli* susceptibility to cefotaxime was 57.14%. *Pseudomonas aeruginosa* showed 100% susceptibility to imipenem. None of the isolates were Extended spectrum beta–lactamase (ESBL) producer. Streptococci was not susceptible to penicillin but was susceptible to vancomycin and linezolid. No Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Intermediate *Staphylococcus aureus* (VISA), Vancomycin Resistant *Staphylococcus aureus* (VRSA) and Vancomycin Resistant *Enterococci* (VRE) were detected.

Duration of hospital stay of < 4 days was found to be statistically significant (p = 0.0000001) in SBP patients as compared to non- SBP patients. All SBP patients with < 4 days hospital stay expired. Duration of hospital stay of < 4 days was statistically significant in Classic SBP as compared to CNNA and MNB cases. All MNB cases had duration of hospital stay of ≥ 4 days.
Complications were seen in 41% patients, of which hepatic encephalopathy was the commonest (38%). In SBP and non - SBP cases, complications were 51.72 % (30/58) and 26.19% (11/42) respectively. Major complication seen in SBP cases was also hepatic encephalopathy, seen in 46.55% (27/58) cases. Maximum complications were seen in CNNA (83.33%), followed by Classic SBP (67.74%).

Overall mortality was 24 %. In SBP cases, mortality was 34.49% (20/58). Mortality in SBP cases was statistically significant in comparison to non-SBP cases (p = 0.003922). Highest mortality was seen in CNNA (66.67%), followed by Classic SBP (45.16%).

Table 1: Different bacteria grown by the three methods

Bacteria	Conventional Method		Direct bedside inoculation in TSB	
	No.	%	No.	%
Escherichia coli	11	57.89	21	40.38
Pseudomonas aeruginosa	4	21.07	6	11.54
Acinetobacter species	1	05.26	6	11.54
Enterobacter species	1	05.26	5	09.62
Proteus mirabilis	1	05.26	2	03.85
Klebsiella pneumoniae	0	00.00	1	01.92
Enterococcus species	0	00.00	4	07.69
Streptococcus species	1	05.26	4	07.69
Methicillin Sensitive *Staphylococcus aureus*	0	00.00	3	05.77
Total	**19**	**100**	**52**	**100**

Table 2: Different bacteria grown by both the methods in Classic SBP and MNB cases

Bacteria	Conventional Method		Direct bedside inoculation in TSB	
	Classic SBP	MNB	Classic SBP	MNB
	No. (%)	No. (%)	No. (%)	No. (%)
Escherichia coli	10 (76.93)	1 (16.67)	18 (58.06)	3 (14.29)
Pseudomonas aeruginosa	1 (07.69)	3 (50.00)	3 (09.67)	3 (14.29)
Acinetobacter species	1 (07.69)	0	2 (06.45)	4 (19.05)
Enterobacter species	0	1 (16.67)	2 (06.45)	3 (14.29)
Proteus mirabilis	1 (07.69)	0	1 (03.23)	1 (04.75)
Klebsiella pneumoniae	0	0	1 (03.23)	0
Enterococcus species	0	0	2 (06.45)	2 (09.52)
Streptococcus species	0	1 (16.67)	1 (03.23)	3 (14.29)
Methicillin Sensitive *Staphylococcus aureus*	0	0	1 (03.23)	2 (09.52)
Total	**13**	**6**	**31**	**21**
	19		**52**	

Table 3: Biochemical parameters in variants of SBP

Variants of SBP	Classic SBP	MNB	CNNA
Ascitic fluid Polymorphonuclear (PMN) count /mm^3	1096.33 ± 812.73	82.73 ± 70.56	828.86 ± 582.00
Ascitic fluid protein level (g/dl)	1.99 ± 1.00	1.59 ± 0.72	2.23 ± 1.13
Serum bilirubin (mg/dl)	5.51 ± 4.76	3.85 ± 4.45	16.7 ± 14.4
Serum creatinine (mg/dl)	1.80 ± 1.02	1.18 ± 0.76	2.87 ± 4.00

Table 4: Susceptibility of bacteria to three groups of antimicrobial agents

Bacteria	Antimicrobial agents					
	Aminoglycosides		Quinolones		Cephalosporins	
	No. susceptible	Total no. tested	No. susceptible	Total no. tested	No. susceptible	Total no. tested
Gram positive cocci	09	11	06	07	05	07
Enterobacteriaceae + *Acinetobacter* species	35	35	28	35	19	35
Pseudomonas aeruginosa	04	06	04	06	04	06
Total (%)	**48 (92.31)**	**52**	**38 (79.17)**	**48**	**28 (58.33)**	**48**

DISCUSSION

Youngest patient in the study was 21 years old and the oldest was 63 years old. Maximum number of cases of cirrhosis with ascites was seen in the age group of 41-60 years (59%). This was almost similar to a study from Nepal where 56% were in the age group between 40-59 years.[16] In 58 cases of SBP, males predominated (89.66%). In the Nepal study, amongst the SBP cases, 94% were males.[16]

The commonest etiology in these patients with cirrhosis was alcoholism (90%) – 85% had history of only alcoholism and 5% were alcoholics and HBs antigen reactive. A similar study from this part of India had also shown alcoholism as the main etiology (57.14%).[11] A recent Asian study also have reported alcohol related cirrhosis in 97.53% cases, while only 2.4% were non-alcoholic.[7]

In the present study, spontaneous bacterial peritonitis (SBP) was present in 58% of cases and the remaining 42% were non-SBP cases. This is similar to the studies reported from Peshawar and Larkana in 2010 (58% and 54% respectively).[8,17] The probable reasons for higher prevalence in the present study and latter studies may be late referral to the tertiary care hospital, low socio

economic conditions and malnutrition.[17] In this study, Classic SBP was commonest (53.45%), followed by 36.21% of MNB. CNNA encountered was only 10.34%. However the study from Larkana did not have any case of MNB, but prevalence of CNNA was 46% .[8] A recent study from India in 2010 have reported Classic SBP in 66.67% cases, CNNA in 25% and MNB in 8.33%,[5] which is almost similar to this study.

Gram negative bacilli (GNB) predominated – 94.74% by conventional method and 78.85% by direct inoculation in TSB. Almost all the studies of ascitic fluid cultures have shown predominance of Gram negative bacilli ranging from 28.17% to 100%.[5,6,7,8,9,16-18] Amongst the Gram negative bacilli, *Escherichia coli* was the commonest bacteria – 57.89% and 40.38%, followed by *Pseudomonas aeruginosa* – 21.07% and 11.54% by conventional method and by direct inoculation in TSB respectively (Table 1). Various studies have isolated *Escherichia coli* from 22.22 to 75% from ascitic fluid.[5,6,7,16,18] *Pseudomonas aeruginosa* and *Acinetobacter species* have also been isolated from ascitic fluid cultures from other studies.[5,6,7,8,9,12,16,17]

Overall 82% of cases were in Child Turcotte Pugh (CTP) class C in this study). In studies from Asian countries, 85% to 88% of their cases belonged to CTP class C.[7,16] In a study from Wardha, all belonged to CTP class C (100%).[6] In this study, fever and pain in abdomen was present in 68.97% and 82.76% patients of SBP respectively and jaundice was present in 82.76% patients. A recent Indian study have reported abdominal pain, fever, jaundice and GI bleed in 75%, 62.5%, 62.5% and 25% cases of SBP respectively.[5]

Total leucocyte count (TLC) in this study was (mean ± SD) 11331.1 ± 7542.15 / mm^3 in SBP cases, which correlated well with a study from Nepal (12580 ± 6564 / mm^3).[7] Serum protein and albumin levels in SBP cases in this study, is almost similar to a study by Syed et al, where the respective values were (mean ± SD) 6.18 ± 1.32 g/dl and 2.22 ± 0.41 g/dl. Even studies from Karnataka and Mumbai have reported serum albumin (mean ± SD) of 2.46 ± 0.54 g/dl and 1.98 ± 0.2 g/dl respectively.[5,11]

In this study, serum bilirubin was comparatively higher in the SBP cases than the non-SBP cases, but this was not statistically significant. Even Doddamani et al and Amrapurkar et al from India have reported serum bilirubin of 6.87 mg/dl and 6.80 ± 5.5 mg/dl respectively in SBP cases.[5,11]

In this study, serum creatinine in SBP cases was 1.73 ± 1.67 mg/dl, which was statistically significant when compared to non-SBP cases. Two studies from Nepal have reported serum creatinine values of 1.57 ± 1.36 mg/dl and 1.60 ± 0.7 mg/dl in the SBP group and they also showed statistical significance in cases of SBP.[7,16]

Ascitic fluid PMN count in this study was statistically significant in SBP cases as compared to non-SBP cases. Other studies also have shown a high ascitic fluid PMN count in SBP cases to the range of (mean ± SD) 785.5 ± 726.4 /mm^3 to 2052.41 ± 206.83 /mm^3. All the studies between the 2 groups were of statistical significance.[7,16-18] Ascitic fluid PMN count of > 250/ mm^3 should be considered as diagnostic of SBP.[19] This count was found to be of statistical significance between Classic SBP and MNB (p= 0.0000001) and also between CNNA and MNB (P= 0.02585), but it was not statistically significant between Classic SBP and CNNA (p = 0.4494). Runyon and Hoefs showed that patients with CNNA behave similar to those with Classic SBP[20] and in this study also PMN counts of Classic SBP and CNNA (mean ± SD) were 1096.33 ± 812.73 /mm^3 and 828.86 ± 582.0 /mm^3 respectively. Ascitic fluid total leucocyte count (mean ± SD) was 1044.40 ± 1013.26 / mm^3 in SBP cases and was statistically significant in comparison to non-SBP cases, which is similar to other Asian studies.[7,21,22]

Overall aminoglycoside susceptibility in this study was 92.31%, followed by 79.17% susceptibility to quinolones and 58.33% to cephalosporins. In a recent report from Egypt,

cefotaxime susceptibility was 29.11% and ciprofloxacin susceptibility was 17.72%.[18] Ciprofloxacin resistance from 2002 to 2005 in a Korean study was 18%,[23] which is in accordance to this study (20%), whereas cefotaxime resistance during the same period in their study was 14.8% and in the present study it is 41.67%.

Amoxycillin-clavulanic acid susceptibility was reported less in the study from Egypt (20.25%), as compared to this study (42.86%) in Gram negative bacilli. The present study shows only 54.29% susceptibility of Gram negative bacilli to cefotaxime. Even the predominant isolate *Escherichia coli* showed susceptibility of only 57.14% to the same antibiotic. Therefore, it is important for the clinicians to be aware of the recent increase in cefotaxime resistance in Gram negative isolates from SBP cases.[23] Though cefotaxime remains the drug of choice for treatment of SBP, the resistance of Gram negative isolates to cefotaxime as reported by the present study is 45.71%. In view of this, the clinicians should be made aware that even if they start with cefotaxime, they should change over to other antimicrobials like quinolones and aminoglycosides, according to the antimicrobial susceptibility report from the laboratory.

Hepatic encephalopathy as a major complication was also seen in studies from Lahore (40%)[24] and Larkana (32%),[8] which are almost similar to the present study (38%). Overall mortality in this study was 24%, which is exactly similar to a study from Portugal (24%).[25] Mortality in the range of 21-28% have been reported by an Indian study[6] and another Asian study.[12]

CONCLUSION

Spontaneous Bacterial Peritonitis (SBP) is a serious complication in patients of advanced cirrhosis with ascites. It is still a highly relevant condition in clinical practice due to its high prevalence, high recurrence rate and poor long term prognosis. Hence, a diagnostic paracentesis should always be performed routinely within 24 hours of admission and ascitic fluid sample should be sent for culture, estimation of cell count and biochemical parameters. This is required to initiate prompt management in these patients so as to reduce mortality and achieve a better survival rate. Direct bedside inoculation in TSB is a superior method of ascitic fluid culture than the conventional method for the diagnosis of SBP. Clinicians should be aware of increased resistance of bacteria to cefotaxime. Ciprofloxacin could be a promising alternative antimicrobial in management of SBP.

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