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Original Research Article

Prospective study of bedside inoculation of blood culture bottles with ascitic fluid versus delayed inoculation for the detection of spontaneous bacterial peritonitis

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ABSTRACT

Background: Spontaneous bacterial peritonitis (SBP) is the most common bacterial infection in cirrhosis, accounting for 10%-30% of all reported bacterial infections in the patients admitted to hospital. Spontaneous bacterial peritonitis (SBP) is the most frequent and life-threatening infection in patients with liver cirrhosis. All forms of cirrhosis have been reported to be complicated by SBP. A delay in the time period between the collection of the ascitic fluid sample, and its inoculation into the blood culture media, has been one of the reasons implicated to account for low-test positivity. There was lack of studies for comparing the bacterial yield between bedside inoculated blood culture bottles with ascitic fluid over delayed inoculation in the detection of SBP. Hence this study is done to compare the bacterial yield between bedside inoculated blood culture bottles with ascitic fluid over delayed inoculation for the detection of SBP.
Methods: Cross sectional study.
Results: Maximum number of cases of cirrhosis with ascites with SBP was seen in the age group of 31-40years (54.4%) with mean age of study population being 39.66years, more common in males, bed side inoculation yielded more positive culture reports compared to delayed inoculation and *E. coli* and *klebsilla* being the common organisms.
Conclusions: Difference between 2 culture methods in isolating organism in SBP cases was not statically significant. But, among culture positive cases, this study demonstrates that bedside inoculation of blood culture bottles is superior to delayed laboratory inoculation.

Keywords: Ascites, Culture sensitivity, Inoculation, Spontaneous bacterial peritonitis

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is the most frequent and life-threatening infection in patients with liver cirrhosis, requiring prompt recognition and treatment. It is defined by the presence of ≥250 polymorphonuclear cells (PMN)/mm^3 in ascites in the absence of an intra-abdominal source of infection or malignancy. It is the most common bacterial infection in cirrhosis, accounting for 10-30% of all reported bacterial infections in the patients admitted to hospital.[1] SBP occurs only in the setting of liver disease, usually in severe liver disease.[2] The liver disease may be chronic, as in alcoholic cirrhosis, subacute, as in alcoholic hepatitis, or acute, as in fulminant hepatic failure.

Currently available evidence suggests that the spontaneous ascitic fluid infection are the result of overgrowth of a specific organism in the gut, translocation of that microbe from the gut to mesenteric

lymph nodes, and resulting spontaneous bacteremia and subsequent colonization of susceptible ascitic fluid.[3]

Although 87% of patients with spontaneous bacterial peritonitis are symptomatic at the time the infection is diagnosed, the symptoms and signs of infection are often subtle, such as a slight change in mental status.[4] Without prompt paracentesis, the diagnosis and treatment of infected ascites may be delayed, often resulting in the death of the patient.

Approximately 80% of SBP is caused by E coli, streptococci (mostly pneumococci), and *Klebsiella*. Anaerobes cause approximately 1% of SBP. Many reported cases of anaerobic SBP are polymicrobial and probably represent misdiagnosed secondary peritonitis.[5]

Risk factors for spontaneous bacterial peritonitis include decreased hepatic synthetic function with associated low total protein level or prolonged prothrombin time (PT) and decompensated state.

All patients suspected of having spontaneous bacterial peritonitis (SBP) must undergo peritoneal fluid analysis. Examination of ascitic fluid for SBP has routinely involved sending the fluid for cell count, differential count, and culture.

The sensitivity of microbiologic studies has been reported to increase significantly with the direct inoculation of routine blood culture bottles at the bedside with 10ml of ascitic fluid. A delay in the time period between the collection of the ascitic fluid sample, and its inoculation into the blood culture media, has been one of the reasons implicated to account for this low-test positivity. Hence study is done to compare the bacterial yield between bedside inoculated blood culture bottles with ascitic fluid over delayed inoculation for the detection of SBP.

Aims and objectives of the study are to evaluate the yield of microbiological cultures in patients with Spontaneous Bacterial Peritonitis.

METHODS

Study design

Cross sectional study

Duration of study

The duration of the study was 2 (two) years, starting from October 2013.

Study population

Chronic liver disease patients with ascites, suspected of having SBP (Spontaneous bacterial peritonitis) were subjects of the study.

Inclusion criteria

- Chronic liver disease patients with ascites suspected to have SBP based on the ascitic fluid analysis, characterised by PMNs count >250cells/ micro litre,
- Worsening or unexplained encephalopathy in cirrhotic patients,
- Worsening or new onset renal failure in cirrhotic patients.

Exclusion criteria

- Patients with suspected secondary peritonitis as in perforated viscus, perinephric abscess and acute appendicitis,
- Indwelling peritoneal catheter,
- Late stage (SBP) resolving infection,
- Patients with urinary tract infection.

Statistical analysis

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ±SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance.

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS

The study was carried out on 90 cases of SBP admitted in Medicine ward/OPD, liver clinic RIMS Imphal.

Of the total 90 patients 84 were male and 6 were female with M: F ratio of 14:1 as shown in Table 1.

Table 1: Gender distribution of patients studied.

Gender	No. of patients	%
Male	84	93.3
Female	6	6.7
Total	90	100

Table 2 shows that 54.4% of the patients were between age 31 to 40 followed by 41 to 50 years which accounted for 34.4% of cases. Mean age of study population was 39.66 years with a standard deviation of 6.91 years.

Table 3 shows, all the patients of cirrhosis with ascites 71.1% were of alcoholic etiology and 81.1% were both alcohol as well as viral with remaining etiologies constituted by Hep B, Hep C, Hep B and C.

Table 2: Age distribution.

Age	No. of patients	%
21-30	5	5.6
31-40	49	54.4
41-50	31	34.4
51-60	4	4.4
>60	1	1.1
Total	90	100

Table 3: Etiology of liver cirrhosis with ascites.

Etiology	No. of patients	%
Alcohol	64	71.1
Alcohol and viral combined	73	81.1
Hep B	9	10
Hep B and C	5	5.6
Hep C	12	13.3

Table 4 shows 81.1% of patients had normal range of serum protein, 11.1% had hypoproteinaemia with mean of 6.75 and SD of 1.09. 83.3% of SBP patients were hypoalbuminemic and 16.7% with values in range of 3-3.5g with mean of 2.37 and SD 0.49. 90% of the SBP patients had S.globulin >3.6g% and 10% patients in the range of 1.8-3.6 with a mean of 4.37 and SD of 0.78.

Table 4: LFT parameters in SBP patients.

LFT parameter	No. of patients(n=90)	%	Mean ±SD
Serum protein (g %)			
<5	10	11.1	6.75±1.09
5-8	73	81.1	
>8	7	7.8	
Serum albumin (g %)			
<3	75	83.3	
3-3.5	15	16.7	2.37±0.49
>3.5	0	0	
Serum globulin (g%)			
<1.8	0	0	
1.8-3.6	9	10	4.37±0.78
>3.6	81	90	

From Table 5 it is observed that 54.4% of patients were having ascetic fluid neutrophil count of 250-500 and around 40% and 5.6% with 500-1000 and >1000 asitic fluid neutrophils respectively. 70% of patients were having ascetic fluid protein <1g% and only 30% with ≥1g%. 97.8% of patients were having ascetic fluid albumin in range of 0.4-1mg% and only 2.2% with >1mg%.

From Table 6 it is observed that ascetic fluid culture was negative in 72.2% of cases when done immediately at bedside where as it was negative in 91.1% when done after a delay, change observed being 18.9%. Ascitic fluid culture yield is significantly reduced when culture is done after a delay with P<0.001**(Paired proportion test).

As shown in Table 7, when culture results were correlated, both with respect to total number of culture positivity and *E. coli* positivity it was statistically significant (P<0.001) in immediately done culture method than that done after a delay.

With respect to *Klebsiella* positivity it was not statistically significant between 2 methods, no significance was observed between 2 methods with respect to Streptococcus pneumoniae.

Table 5: Ascitic Fluid analysis.

	No. of patients (N=90)	%	Mean ±SD
A.F. Neutrophil Count (perμL)			
250-500	49	54.4	591.3±273.9
500-1000	36	40	
>1000	5	5.6	
A. F. Protein (G%)			
<1	63	70	1.04±0.3418
≥1	27	30	
A. F. Albumin (G%)			
0.4-1	88	97.8	0.43±0.0862
>1	2	2.2	

Table 6: Ascitic fluid culture yield.

A. F. culture yield	Immediate (at bedside) (%)	After a delay (%)	% change
Negative	65 (72.2)	82 (91.1)	+18.9
E. coli	18 (20)	7 (7.8)	-12.2
Klebsiella	4 (4.4)	1 (1.1)	-3.3
Streptococcus pneumoniae	3 (3.3)	0 (0)	-3.3
Total	90 (100)	90 (100)	-

DISCUSSION

Spontaneous Bacterial Peritonitis (SBP) is the most common, potentially fatal, yet reversible cause of deterioration in patients with advanced cirrhosis with ascites. Maximum number of cases of cirrhosis with ascites with SBP was seen in the age group of 31-40 years (54.4%) with mean age of study population being 39.66years with a standard deviation of 6.91years. But in the study conducted by Filik L et al the mean age of all patients was 49.91+/-15.01years.[6]

Contrary may be because of impurity of alcohol consumed and age of intake of alcohol in our study population. Higher percentage (93.3%) of males as cases was closely in comparison to Filik L et al study where in males were 69.6% and females were 30.4%.[6] Slight difference observed may be because of preponderance of alcoholism in male population. The commonest aetiology for cirrhosis in the study population was alcoholism (71.1%) and 81.1% had combined etiology i.e.

alcoholism and viral etiology (HBV, HCV). This was comparable with study conducted by Bankar SS et al in which alcoholism (84.48%) was the commonest etiology for cirrhosis in SBP cases.[7]

Table 7: Correlation of AF culture immediately done at bedside and AF culture after a delay.

AF culture immediately done at bedside	AF culture after a delay				
	Negative (%)	***E. coli (%)***	***Klebsiella (%)***	**Total (%)**	**P value**
Negative	65 (79.2)	0 (0)	0 (0)	65 (72.3)	<0.001**
E. coli	11(13.4)	7 (100)	0 (0)	18 (20)	<0.001**
Klebsiella	3 (3.7)	0 (0)	1 (100)	4 (4.4)	0.073+
Streptococcus pneumoniae	3 (3.7)	0 (0)	0 (0)	3 (3.3)	1.000
Total	82 (100)	7 (100)	1 (100)	90 (100)	-

In this study, ascetic fluid PMN count was (mean ±SD) 591.3±273.96 per mm^3 and that of ascetic protein was (mean ±SD) 1.041±0.0862g%. Ascetic mean PMN count was low when compared to study conducted by Bankar S et al where in mean ascitic fluid polymorphonuclear count (PMN) count was 755.19±788.04/mm^3.[3-7]

In 25 episodes in which the bedside bottles were culture positive, only in 8 episodes by the delayed culture method demonstrated growth; this difference was statistically significant ($P < 0.001$). According to study conducted by Runyon BA et al, in 29 episodes in which the bedside bottles were culture positive, only 22 (75.9%) of the laboratory inoculated sets demonstrated growth; this difference was statistically significant ($P < 0.02$).[8]

Among culture positive cases, Gram negative bacilli (*E. coli* and *Klebsiella*) predominated in 88% of cases while 12% were Gram positive (*S. pneumoniae*) by immediate culture method (done at bedside) whereas it was 87.5% and 12.5% by delayed culture method. Amongst the Gram-negative bacilli, *Escherichia coli* was the commonest bacteria-78.6% and followed by *Klebsiella*-21.4%.

It was comparable to studies conducted by others. According to a study conducted by Pawar GP et al, *Escherichia coli* was the commonest organism cultured, being found in 60%.[9] According to a study conducted by Bibi S et al, *E. coli* (65%) was the predominant pathogen followed by Enterococcus species (15%).[10]

CONCLUSION

Overall, the difference between 2 culture methods in isolating organism in SBP cases was not statically significant. But, among culture positive cases, this study demonstrates that bedside inoculation of blood culture bottles is superior to delayed laboratory inoculation of blood culture bottles in the detection of bacterial growth in spontaneous bacterial peritonitis.

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