

Ascitic Fluid Culture in Cirrhotic Patients with Spontaneous Bacterial Peritonitis

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ABSTRACT

Objective: To determine the frequency and compare the culture yield of bacterial isolation by conventional and blood culture BACTEC bottle techniques in cirrhotic patients with spontaneous bacterial peritonitis (SBP).

Study Design: Cross-sectional comparative study.

Place and Duration of Study: Pathology Department, Bannu Medical College, Bannu, KPK, from January 2012 to December 2013.

Methodology: Paracentesis of 20 ml of ascitic fluid tapped from cirrhotic patients with SBP was carried out by a single technologist. The analysis included differential leukocyte count (DLC), while 5 ml each of the fluid was inoculated into conventional culture media and BACTEC blood culture bottle. All the data were analysed on (SPSS) version 16 to determine frequencies with percentages and mean values with standard deviation. Chi-square test was used for comparing the yield of conventional and blood culture bottle methods. P-value was considered significant if < 0.05 .

Results: In 105 cases of ascitic fluid analyses, 27 (25.72%) had positive ascitic fluid culture whereas 78 (74.28%) had negative ascitic fluid culture. Ascitic fluid culture was positive in 6 cases by conventional culture media and in 27 cases by BACTEC culture bottle media ($p < 0.001$). Bacterial isolation was obtained by both culture methods in 6 cases ($p < 0.001$).

Conclusion: Direct bedside inoculation of ascitic fluid by BACTEC culture bottle method has better yield as compared to conventional culture method.

Key Words: Spontaneous bacterial peritonitis. Cirrhosis. Ascites. Ascitic fluid culture. Conventional culture method. BACTEC culture bottle method.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is the bacterial infection of the abdominal cavity in absence of any obvious source of infection, either having positive bacterial culture or neutrophil count $\geq 250/\text{ml}$.¹ Bacterial culture almost invariably yields a single growth in SBP. The presence of more than one organism suggests secondary peritonitis. Other variant of peritonitis like culture-negative neutrocytic ascites (CNNA), as diagnosed based on an increased neutrophils count although culture is negative. Still another variant of SBP is called monomicrobial non-neutrocytic bacterascites in which ascitic fluid culture is positive but neutrophils count is less than 250/cmm.² SBP occurs almost exclusively in patients with portal hypertension, usually as a result of cirrhosis of the liver. Generally the source of the infectious agent is not easily identifiable.³ Clinical findings include fever, chills, nausea, vomiting, abdominal pain, tenderness and general malaise. Pathophysiology of the SBP is not clearly understood. The likely cause may be seeding of bacteria and their endotoxins from gastrointestinal tract (GIT) to

peritoneal cavity due to impaired defensive mechanisms in cirrhosis; bacteremia from urinary tract or respiratory tract can be another source of infection of SBP or iatrogenic cases, like endoscopic treatment of esophageal varices or gastric varices may be a source of infection causing peritonitis.⁴

In secondary bacterial peritonitis, culture is positive but usually contains more than one microorganism; and the neutrophils count is equal to or more than 250/cmm, here a source of infection is present. Clinical features do not distinguish SBP from secondary bacterial peritonitis.^{5,6}

Patients with chronic liver disease, who develop cirrhosis, represent a group susceptible to several complications during the course of the disease including SBP with high morbidity and mortality.⁷ The prevalence of spontaneous bacterial peritonitis ranges between 10% and 30%. Its diagnosis is based on laboratory tests of the ascitic fluid obtained by paracentesis.^{1,8} It is more likely to be a bacteremia in terms of the numbers of bacteria present.^{9,10} The conventional method of culture has been found to detect bacterial growth in approximately 50% of neutrocytic samples, whereas bedside inoculation of blood culture bottles with ascitic fluid detects growth in up to 80%.^{1,11} *Eschrechia coli*, *Streptococci* (mostly *Pneumococci*) and *Klebseila* are the most frequently isolated microorganisms (60%).¹²

Internationally there is a growing awareness about the influence of the method of culture on the yield of culture of ascitic fluid.¹³

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The aim of this study was to determine the frequency and compare the culture yield by conventional culture as well as BACTEC culture bottle method in cirrhotic patients suffering from SBP.

METHODOLOGY

This descriptive cross-sectional comparative study was conducted at the Pathology Department of Bannu Medical College, Bannu, KPK, Pakistan, from January 2012 to December 2013. The sample size was 105 ascitic fluid specimen. Inclusion criteria were all cirrhotic patients of either gender and all ages who presented with clinical features of spontaneous bacterial peritonitis. Exclusion criteria were peritonitis due to any other cause or patients having taken treatment or have undergone paracentesis in the previous month. After informed verbal consent, patients were assessed by history, physical examination, and ancillary investigations which included abdominal ultrasonography, paracentesis for ascitic fluid, differential leukocyte count (DLC), culture analysis in all cases, enzyme linked immunosorbant assay (ELISA), and polymerase chain reaction (PCR) for hepatitis B and C. Paracentesis of 20 ml of ascitic fluid was carried out by a single technologist and the laboratory analysis was carried out. Ten ml of ascitic fluid was used for routine examination including total and differential leukocyte count and 5 ml each was inoculated in conventional culture media, and BACTEC blood culture bottle media to compare bacterial yield by either method.

All the data were collected on a designed proforma and analysed by a Statistical Package for Social Sciences (SPSS) version 16 to measure frequencies with percentages and mean with standard deviation where applicable. All parameters of interest were tested by chi-square test. P-value < 0.05 was considered as significant.

RESULTS

In 105 ascitic fluid samples, 68 (64.76%) were from male and 37 (35.24%) from female patients with male to female ratio of 1.37:1. The age ranged from 41 - 80 years with mean age of 51 ±10 years. Most of the patients, 40 (38.09%), were in the age group of 51 - 60 years followed by 36 (32.28%) patients in the age group of 41 - 50 years. Twenty-seven (25.71%) ascitic fluid cultures were positive and 78 (72.24%) were negative. Six (5.71%) ascitic fluid cultures were positive by conventional culture method (p < 0.001) and 27 (25.71%) ascitic fluid culture by BACTEC culture bottle method (p < 0.001). Bacterial isolation was obtained by both methods in 6 patients (p < 0.001, Table I).

In 69 (65.71%) patients, absolute polymorphonuclear count was > 250 cells/cmm, < 250 cells/cmm in 35 (33.33%) and equal to 250 cells/cmm in only one (0.95%) case.

Table I: Results of ascitic fluid by BACTEC and conventional culture methods (n=105).

Culture method	Positive cases	Negative cases	p-value
BACTEC	27 (25.72%)	78 (74.28%)	<0.001
Conventional	06 (5.71%)	99 (94.28%)	<0.001
Both methods	06 (5.71%)	99 (94.28%)	<0.001

Table II: Frequency of bacteria present in culture positive ascitic fluid (n=27).

Type of bacteria	Positive culture	Percentages
<i>E. coli</i>	16	59.25%
<i>Pseudomonas</i>	06	22.22%
<i>Klebsiella</i>	03	11.11%
<i>Staphylococcus aureus</i>	01	3.70%
<i>Streptococcus epidermidis</i>	01	3.70%
Total	27	100%

Majority of the patients 64 (60.92%) were hepatitis C positive, 18 (17.14%) were hepatitis B positive and 23 (21.90%) patients were negative for both HCV and HBV markers, but they had ultrasonographic evidence of decompensated liver disease.

In 27 positive ascitic fluid cultures, Gram negative bacilli were present in 25 (92.59%) cases and Gram positive cocci in 2 (7.41%) cases. Amongst the Gram negative bacilli, *E. coli* was present in 16 (59.25%) cases followed by *Pseudomonas* and *Klebsiella* in 06 (22.22%) and 03 (11.11%) cases, respectively. Amongst the Gram positive cocci, there was one case each (3.70%) of *Staphylococcus aureus* and *Streptococcus epidermidis* (Table II).

DISCUSSION

Spontaneous bacterial peritonitis (SBP) is defined as infection of the previously sterile ascitic fluid without any apparent intra-abdominal source of infection.¹ The diagnostic criteria for SBP is the presence of > 500/cmm leukocytes or the presence of > 250/cmm neutrophils in the ascitic fluid and or a positive ascitic fluid culture. Spontaneous bacterial peritonitis is one of the common complications of cirrhosis with a frequency ranging from 7% to 23%.^{14,15}

In this study, the age ranged from 41 to 80 years with a mean age of 50 ±10 years and the male to female ratio was 1.37:1. In a study conducted by Olademji *et al.*, the age range was 43 - 78 years with a male to female ratio of 1.2:1.³ In this study, the common age group of disease presentation was 51 - 60 years, followed by 41 - 50 years, whereas in the study by Olademji *et al.*, it was 61- 80 years followed by 41 - 60 years.³

In this study, the frequency of SBP was 25.71% whereas Amarapurkar *et al.* reported 22% incidence, Obstein *et al.* reported 26.12%, and Japson *et al.* reported 27% incidence.⁴⁻⁶ Other studies show a quite higher incidences reported as 38%, 38.23%, 38%, 35.4% and

34.92%, respectively.^{7-10,19} Still other studies show a much higher incidence of SBT reported as 67.7% and 56.25% by Oladimeji and Memon *et al.*, respectively.^{3,18}

The most common group of bacteria isolated in this study was Gram negative bacilli, followed by Gram positive cocci. Amongst the Gram negative bacilli, *Escherichia coli* (59.25%) was the commonest bacteria followed by *Pseudomonas* (22.22%) and *Klebsiella* (11.11%). Amongst the Gram positive cocci, *Staphylococci* and *Streptococci* were 3.70% each. Olademji *et al.* reported Gram negative bacilli as 66.7% and Gram positive cocci as 33.3%.³ Here the commonest bacteria was *E. coli* too, followed by *Klebsiella*, *Streptococci* and *Staphylococci*. De *et al.* reported Gram negative bacilli as 78%, amongst which *E. coli* (40%) was the commonest bacteria followed by *Pseudomonas aeruginosa* and *Acinetobacter* (11%) each. Amongst the Gram positive cocci, *Streptococci* (7%) was the commonest bacterium.¹ Gill *et al.* also showed *E. coli* (70%) as the commonest Gram negative bacilli followed by *Klebsiella*.¹¹ Iqbal *et al.* reported from Khyber Teaching Hospital, Peshawar that *E. coli* were 58.13% in SBP ascites followed by *Streptococcus pneumoniae* in 18.60%, *Staphylococcus aureus* in 9.13% and *Acinetobacter* in 4.63% cases.⁸ Haider *et al.* found 60% of the cultured bacteria as Gram negative bacilli and 24% as Gram positive cocci, amongst these *E. coli* were 30%, *Klebsiella* 14% and *Enterobacter* and *Pseudomonas* 4% each.²¹

This study showed 64 (60.92%) patients of hepatitis C, 18 (17.14%) patients of hepatitis B and 23 (21.90%) patients negative for both HCV and HBV markers. Zaman *et al.* reported 54% HCV positive cases, 20% HBV positive cases, 10% combined HCV and HBV cases and 16% cases were negative for both HCV and HBV infections.² Mehar *et al.* reported HCV in 67.34% cases and HBV in 16.32% cases.²²

Ascitic fluid culture method greatly influenced bacterial isolation in this study. Out of 105 patients, ascitic fluid inoculation in bedside BACTEC blood culture bottle resulted in bacterial growth in 27 (25.72 %) cases whereas by conventional method only in 6 (5.71%) cases bacterial culture was positive. De *et al.* reported 55.77% positive culture by direct bedside inoculation of the ascitic fluid and in 36.54% cases by conventional method.¹ Bobadilla *et al.* reported positive culture in 35.48% by modified culture method and in 6.45% positive culture by conventional method.²³ Runyon *et al.* also showed the same improved sensitivity of inoculation by blood culture bottle method in two separate studies from 42% to 91%.^{12,14} Castolette *et al.* reported higher sensitivity by direct bedside inoculation.¹⁶ Studies conducted in Asian as well as Western countries also reported superiority of bedside direct inoculation over the conventional method. The

reason of better culture yield by BACTEC culture bottle method may be due to the presence of antiphagocytic and anticomplementary activity in BACTEC blood culture bottle; whereas, the reason of low yield by conventional method may be due to the low concentration of bacteria in ascitic fluid and the gap in time period between the ascitic fluid collection and its inoculation on conventional media and also more time availability for the neutrophils to phagocytose bacteria present in the ascitic fluid.

CONCLUSION

This study showed that direct bedside inoculation by BACTEC culture bottle method has a better yield as compared to conventional culture method.

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