SPONTANEOUS BACTERIAL PERITONITIS;
FREQUENCY OF CULTURE POSITIVE (SBP) AND CULTURE NEGATIVE SPONTANEOUS BACTERIAL PERITONITIS (NEUTROCYTIC ASCITES) IN CIRRHOTIC POPULATION.

Sana Jafri¹, Riaz Hussain Awan², Seema Nayab³, Khadim Hussain Awan⁴

ABSTRACT… Objectives: To determine the frequency of culture positive (SBP) and culture negative spontaneous bacterial peritonitis (neutrocytic ascites) in cirrhotic population. Period: The six months (November 08th 2012 to May 07th 2013). Study Design: Descriptive cross-sectional study. Setting: Department of Gastroenterology at Liaquat National Hospital. Total 107 patients of liver cirrhosis with ascites admitted in our hospital. All the specific patients had ascitic fluid DR and C/S were enrolled and evaluated. Patient’s information was recorded on proforma and analyzed by using SPSS-20.0. Results: SBP was detected in 10 7 patients aged between 18 and 67 years included in study. Out of 107 patients with SBP 23 (21.5%) were culture positive while 84 (78.5%) were culture negative. In the culture positive group, 19 (82.6%) were male and 4 (17.4%) were female while in culture negative group 46 (54.76%) were male and 38 (45.24%) were female. The ascitic fluid mean total leukocyte count in patients with culture positive ascites was 5140.39 / mm³ and in culture negative ascites was 2654.26 / mm³. The ascitic fluid mean neutrophils count in subjects with culture positive ascites was 75.57% and in culture negative ascites was 76.02%. The ascitic fluid mean lymphocyte count in individuals had culture positive ascites was 26.09 % and in subjects had culture negative ascites was 23.97%. Conclusion: Frequency of culture negative ascites is greater than culture positive ascites in SBP. The ascitic fluid mean total leucocyte count for culture positive ascites is greater than culture negative ascites. Mortality is high in culture positive ascites.

Key words: Spontaneous, Bacterial, Peritonitis, Liver, Cirrhosis.

INTRODUCTION
Cirrhosis is a very common ailment in Pakistan and ascitic fluid infections (AFI) is the common and life threatening complication of liver cirrhosis as spontaneous bacterial peritonitis (SBP) with polymorph nuclear (PMN) count >250/mm³ and positive ascitic fluid culture, culture negative neutrocytic ascites (CNNA) with PMN > 250/ mm³ with negative ascitic fluid culture, mono or poly microbiral bacterascities with PMN count <250/mm³ and positive ascitic fluid culture with single or multiple pathogen.1-7 When SBP was first described, its mortality exceeded 90% with early diagnosis and prompt treatment.8 A recurrence rate of 70% after one year.9 The prevalence of SBP in Pakistan has been reported to be 32- 64%.6,10 Frequency of culture positive ascites in liver cirrhotic patients with SBP has been described as 23% while 76.4% had CNNA in the reported cases.11 The CNNA group shows low mortality as compared to SBP7,12 Rationale of this study was although SBP is a frequent complication of cirrhosis, by doing culture sensitivity for ascites we could find out the mortality in ascitic fluid infected patients. We believed, that results of this study considered more valid as patients from different economic class, with different causes of liver disease presenting to a big tertiary care hospital of Pakistan will be scrutinized and can better manage as far as preventive strategies in SBP are concerned.

PATIENTS AND METHODS
This six months (November 08th, 2012 to May 07th, 2013) descriptive cross-sectional study was conducted in Liaquat National Hospital Karachi. The sample size were estimated by evaluating the prevalence of SBP in cirrhosis patients was...
found to be 23.5% with culture positive in 31.8%; based on this findings, estimated sample size on 5% bound of error and 95% confidence interval with a prevalence of 23.5 % comes out to be 107.

The inclusion criteria were:
1. Patients with either sex.
2. Age 18-67 years.
3. All patients attending gastroenterology department with ascites due to cirrhosis of liver presenting with either of signs and symptoms of AFI i.e. fever, abdominal pain, tenderness and/or vomiting and tested positive for SBP on Ascitic fluid D/R results.

The exclusion criteria were:
1. Cirrhotic subjects without ascites.
2. Cirrhotic subjects with ascites not tested positive for SBP.
3. Patients who had non-cirrhotic ascites due to secondary bacterial peritonitis, T.B and malignancy.
4. Patients who had received antibiotics in the last 72 hrs.

**Cirrhosis of Liver**
Was diagnosed on the basis of clinical (hepatic encephalopathy, ascites), hematological (platelets count <100,000 prothrombin time >3 seconds of control) and ultrasound findings (i.e. shrunken or altered echo-texture of liver, splenomegaly, increase portal vein diameter) regardless of their duration of illness.

**Hepatic Encephalopathy**
Any patient with diagnosed liver cirrhosis presented with altered mental state i.e confusion, drowsiness, increased sleep, or coma (any of these) without any unilateral weakness of body on clinical examination.

**Spontaneous Bacterial Peritonitis**
An elevated ascitic fluid absolute polymorph nuclear neutrophilic leukocyte (PMN) counts (i.e. >250 cells /mm³) with a positive ascitic fluid culture.

**Positive Ascitic Fluid Culture**
When pathogen detected from the ascitic fluid for culture and sensitivity.

**Culture-Negative Neutrocytic Ascites**
PMN count ≥250 cells/mm³ with ascitic fluid culture negative.

**Monomicrobial Non-Neutrocytic Bacterascites**
PMN count < 250 cells/mm³ with ascitic fluid culture positive with single pathogen.

**Polymicrobial Bacterascites**
PMN count (<250 cells/mm³) with a positive ascitic fluid culture with multiple pathogens.

Patients fulfilled the inclusion criteria were included in this study. After explaining about the study purpose an informed consent was taken from the patient included in this study. Patient’s history and physical examination was conducted by the principal investigator. After thorough clinical evaluation for ascities, sample for ascitic fluid DR were performed by the principal investigator and was sent to Laboratory of LNH. On the results of ascitic fluid DR, patients having SBP were selected for Ascitic Fluid Culture and Sensitivity Test. After asceptic measures the ascitic fluid in 10 CC syringe was collected and sent to laboratory for analysis. All investigations was performed in the well equipped laboratory of Liaquat National Hospital had automated cell counters and skilled expertise. Physical findings and laboratory results were marked on a pre designed Performa comprising of physical findings, symptoms, marking of laboratory findings and outcome of ascities fluid culture. Confounding variables as well as bias were controlled by strictly following the exclusion. The data will be entered and analyzed through Statistical Package for Social Sciences (SPSS) Version 20. Mean and standard deviation, frequencies & percentages were computed while the chi-square test was used and the P-value ≤ 0.05 was taken as significant.

**RESULTS**
A total of 107 patients were included in the study. It included 65 (60.7%) male patients and 42 (39.3%) female patients with age range of 18-67years mean age was 54.13 years (SD ± 7.313). Out of 107 patients with SBP 23 (21.5%)
were culture positive while 84 (78.5%) were culture negative. In the culture positive group, 19 (82.6%) were male and 4 (17.4%) were female while in culture negative group 46 (54.76%) were male and 38 (45.24%) were female. 3 patients had age between 18-30yrs (all 3 culture negative), 12 patients were aged between 31-50 yrs (1 culture positive and 11 culture negative) and 92 patients between 51-70yrs (22 culture positive and 70 culture negative). The results are presented in Figure 1-2 and table 1-2.

Abdominal distension was found in all patients of culture positive and culture negative ascitic groups, while abdominal pain was found in 23 patients of culture positive and 78 patients of culture negative ascites group and no abdominal pain in 6 patients of culture negative group, fever was present in 18 patients of culture positive and in 7 patients of culture negative ascites group. No fever found in 5 patients of culture positive ascites group and 77 patients of culture negative ascites group.

The ascitic fluid mean total leukocyte count in patients with culture positive ascites was 5140.39 / mm$^3$ and in culture negative ascites was 2654.26 / mm$^3$. The ascitic fluid mean neutrophils count in patients with culture positive ascites was 75.57% and in culture negative ascites was 76.02%. The ascitic fluid mean lymphocyte count in subjects had positive ascites culture was 26.09 % and in individuals had negative ascites culture was 23.97%.

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<td>22</td>
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<td>Total</td>
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<td>84</td>
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**Table-I. Age distribution of subjects positive and negative ascitic fluid culture n = 107**

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<td>38</td>
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<tr>
<td>Total</td>
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<td>84</td>
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**Table-II. Frequency of gender with culture positive and culture negative ascites: n = 107**
DISCUSSION
Advances in medical science have resulted in prolonged survival of patients suffering from chronic disease. Infections in patients with liver cirrhosis are frequent, recurrent and associated with unfavorable outcome. Development of ascites in cirrhosis is a multifactorial phenomenon and indicates the severity of underlying disease. Spontaneous ascitic infection is the invasion of ascitic fluid by bacteria without any apparent intra-abdominal septic focus. Mean age in our study was 54.13 years. This means that considerable time elapsed before complication occurred in the underlying disease. Our patients usually develop cirrhosis due to HBV & HCV while alcohol is the commonest cause of cirrhosis in the West. SBP is treatable and requires early diagnosis as most patients are asymptomatic. Diagnosis of SBP is based on clinical suspicion and result of ascitic fluid analysis.

Ascitic fluid analysis includes total and differential leukocyte count and culture. Results of gram staining of smear obtained from ascitic fluid are frequently negative due to low concentration of bacteria in flora, while culture is positive in 50-80% of cases. Due to low sensitivity of staining and culture of ascitic fluid, PMN count in excess of 250 cells/ mm³ is considered adequate for diagnosis and an indication for empirical antibiotics. Conventional ascitic fluid culture methods failed in large number of patients with cirrhotic ascites who otherwise met clinical and PMN count criteria for the diagnosis of spontaneous bacterial peritonitis. Blood culture bottle method of ascitic fluid gives a high positive yield.

In our study, 107 cirrhotic patients with ascites met the diagnostic criteria of SBP and its variants. This yield is lower compared to that of 84%, reported in a local study by Haider and his colleagues. Studies from abroad have reported SBP in 7-25% of cirrhotic patients. The probable reason for such high incidence in Pakistan may be late referral to tertiary care hospitals, poverty, undernourishment, poor hygienic conditions and high prevalence of infectious disease. Immediate and bedside inoculation of blood culture bottles prevents further killing of bacteria by neutrophils present in ascitic fluid and more viable bacteria gave positive culture results, furthermore bacteria immediately found a suitable environment which favored their growth without delay. On the contrary, during transportation of ascitic fluid to the laboratory, neutrophils find enough time to engulf bacteria which reduces the number of viable bacteria in the ascitic fluid which is why delayed inoculation in the laboratory results in negative culture.

The other important factor which enhanced the culture positivity is the large size of bacterial inoculation. Spontaneously infected ascites usually contains low concentration of bacteria. Thus by culturing large amount of ascitic fluid i.e. 10 ml into the blood culture bottles, we inoculate more bacteria, thereby resulting in higher positive yield. In our patients, out of 107 SBP patients 23 (21.5%) were culture positive while 84 (78.5%) were culture negative. The more common presenting symptoms were abdominal pain, abdominal distension and fever. The ascitic fluid mean total leukocyte count in patients with culture positive ascites was 5140.39 / mm³ and in culture negative ascites was 2654.26 / mm³. The ascitic fluid mean neutrophils count in subjects with positive ascites culture was 75.57% and negative ascitic culture was 76.02%. The ascitic fluid mean lymphocyte count in individuals with positive ascitic culture was 26.09 % and in individuals with negative ascitic culture was 23.97%.

CONCLUSION
The study findings demonstrate positive ascitic fluid culture is more common in males. The ascitic fluid mean total leucocyte count for culture positive ascites is greater than culture negative ascites. Most patients with culture positive ascites have fever. Frequency of culture negative ascites is greater than culture positive ascites in SBP. Mortality is high in culture positive ascites. Thus, immediate evaluation and treatment reduced the burden of mortality in cirrhotic population.

REFERENCES


**AUTHORSHIP AND CONTRIBUTION DECLARATION**

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<tr>
<td>1</td>
<td>Sana Jafri</td>
<td>Contribution to conception and design, acquisition of data, analysis and interpretation of data. Drafting the article and shares its expert research opinion and experience in finalizing the manuscript. Contributed in conception and interpretation of data and give his expert view for manuscript designing. Collection and acquisition of data, analysis and interpretation of data and make it suitable for final revision and a corresponding author.</td>
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