Original Article
DOI: https://doi.org/10.70749/ijbr.v3i4.861

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Frequency of Culture-Negative and Culture-Positive Ascites in Chronic Liver Disease Patients Presenting with Spontaneous Bacterial Peritonitis

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ARTICLE INFO

Keywords
Spontaneous Bacterial Peritonitis, Chronic Liver Disease, Ascitic Fluid, Culture-Negative Ascites

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Declaration
Authors' Contribution: *Described at the end of the paper.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History
Received: 01-03-2025, Revised: 22-03-2025
Accepted: 01-04-2025, Published: 20-04-2025

ABSTRACT

Background: Spontaneous bacterial peritonitis (SBP) is a severe complication in chronic liver disease (CLD) patients, characterized by infection of ascitic fluid. The differentiation between culture-positive and culture-negative ascites plays a significant role in understanding the pathology of SBP and ensuring accurate diagnosis and treatment. This study investigates the prevalence of culture-negative and culture-positive ascites in CLD patients with SBP and evaluates the impact of demographic factors on ascitic fluid culture outcomes. **Objective:** To determine the frequency of culture-negative and culture-positive ascites in chronic liver disease patients presenting with spontaneous bacterial peritonitis to a tertiary care hospital. **Study Design:** Cross-sectional study. **Duration and Place of Study:** The study was conducted from November 2024 to February 2025 at the Department of Gastroenterology, PGMI/Shaikh Zayed Hospital, Lahore. **Methodology:** A total of 176 patients aged 30–60 years, diagnosed with CLD and presenting with suspected SBP, were enrolled using a non-probability consecutive sampling method. Ascitic fluid was collected within 24 hours of hospitalization for bacterial culture. **Results:** The mean age of patients was 46.17 ± 8.66 years, with a predominance of males (78.4%). Out of 176 samples, 50 (28.4%) were culture-positive and 126 (71.6%) were culture-negative. Gender significantly influenced culture positivity (p = 0.024), with a higher proportion of males showing positive results (32.6%) compared to females (13.2%). **Conclusion:** Our study concludes that spontaneous bacterial peritonitis with culture-negative ascitic fluid is common in cirrhotic patients. Duration of illness and gender significantly influence culture positivity. These factors should guide early intervention, while ongoing monitoring of microbial resistance and gender-sensitive treatment remain crucial for effective SBP management.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a life-threatening and critical infection in a patient with an underlying disease of the liver,[1] most often in a patient with an underlying disease of the liver, and is defined by bacterial presence in the ascitic fluid in the absence of an apparent source of infection in the intra-abdominal cavity, such as an infected organ or an abscess.[2] SBP is most characteristically gradual in onset with an absence of specific symptoms, such as fever, abdominal pain, and altered mental state, and early diagnosis can therefore become an obstacle.[3] SBP occurs through bacterial translocation from the gut to the peritoneum, with compromised host defenses and gut barrier failure allowing such translocation.[4] Prompt and appropriate antibiotics and early intervention and precise administration of antibiotics are important for an improvement in prognosis, with delayed therapy having a chance of producing complications such as septic shock, renal failure, and even mortality.[5]

Chronic Liver Disease (CLD) consists of disease processes that produce ongoing, long-term hepatic damage.[6] Etiologies include alcoholic and non-alcoholic (e.g., NAFLD, NASH, etc.) and infectious (e.g., hepatitis C and B, etc.) disease, autoimmune disease, and genetic disease (e.g., hemochromatosis, etc.).[7] As disease progresses in CLD, cirrhosis, portal hypertension, and development of ascites, an accumulation of peritoneal fluid, occur.[8] Not only is physiological function and life compromised with ascites, but infection with spontaneous bacterial peritonitis, an progressive infection in such a compromised state, is predisposed to occur.[9] Compromised immune function in CLD then predisposes to infection, and a spiral of deterioration and complications begins.[10]

Culture-Positive Ascites is ascitic fluid, when cultured, will yield microbial growth, indicative of an active infection in the peritoneal cavity.[11] In spontaneous

bacterial peritonitis, a causative organism through a culture is important in guiding targeted therapy with antibiotics.[12] Klebsiella pneumoniae, Escherichia coli, and enteric gram-negative bacilli are prevalent pathogens, but polymicrobial infection can occur in any case.[12] Culture-negative neutrocytic ascites, in which no organism will yield in increased white blood cells, is a challenge in diagnosing.[13] With newer molecular testing, such as polymerase chain reaction (PCR) testing, increased detection rates have become available, allowing for a precise determination of pathogens even when cultures cannot yield them.[14] Prompt intervention according to cultures is important in preventing both morbidity and mortality in such a case.

The clinicopathological and pathophysiological complexity of such a triad is exemplified by spontaneous bacterial peritonitis, cirrhotic hepatic disease, and positive cultures in ascites.[15] Chronic hepatic disease creates a background for an environment for immune dysregulation and the pathogenesis of ascites, and creates a stage for development of SBP in such a background.[16] Once developed, SBP, positive cultures in ascites validate an infectious etiology, and immediate antimicrobial therapy is indicated. Delayed intervention in SBP accelerates liver decompensation, worsens ascites, and predisposes to recurrent SBP.[16]

In a study of cultured samples suspected of SBP, 123 (66.2%) had negative cultures, while 63 (33.8%) showed positive results.[17] A local investigation in Pakistan on patients with CLD found that 50 (39.06%) had classical SBP, 6 (4.68%) had bacterascites, and 72 (56.25%) were diagnosed with Culture Negative Neutrocytic Ascites (CNNA).[18]

Spontaneous bacterial peritonitis remains a significant cause of morbidity and mortality in patients with chronic liver disease. However, distinguishing between culture-negative and culture-positive ascites in these patients presents diagnostic challenges. Culture-negative ascites, despite the absence of bacterial growth, can present similarly to SBP and is associated with poor outcomes. Therefore, it is essential to explore the clinical characteristics, prevalence, and implications of both culture-negative and culture-positive ascites in SBP to improve early diagnosis, therapeutic strategies, and patient prognosis. This study aims to contribute valuable data to guide clinicians in managing SBP in CLD patients more effectively.

METHODOLOGY

This cross-sectional study was conducted between November 2024 and February 2025 at the Department of Gastroenterology, PGMI/Shaikh Zayed Hospital, Lahore. The study aimed to investigate culture-negative and culture-positive ascites in chronic liver disease (CLD) patients with spontaneous bacterial peritonitis (SBP). Based on a 95% confidence level, a 7% margin of error, and an estimated frequency of culture-positive ascites of 33.8%,[17] a sample size of 176 patients was calculated.

The participants were recruited through a non-probability consecutive sampling method. Inclusion criteria consisted of age between 30 and 60 years with a minimum six-month duration of a confirmed case of CLD, with nodularity of liver surface, coarsening of echo texture of the liver, and presence of ascites, confirmed through abdominal ultrasound and physical examination. There must have been spontaneous bacterial peritonitis present in them. Exclusion criteria consisted of non-cirrhotic ascites, current antibiotic use, any severe comorbidities such as diabetes mellitus and kidney disease, and a history of having performed a paracentesis in the previous two weeks.

Upon obtaining informed consent, patients underwent diagnostic paracentesis within 24 hours of hospitalization and prior to antibiotic treatment. Ten milliliters of ascitic fluid were collected and sent for bacterial culture to determine the presence of a causative organism. The presence of culture-negative ascites was defined by the absence of bacterial growth, while culture-positive ascites was confirmed if a causative organism was identified at $\geq 10^5$ / HPF. The diagnosis of spontaneous bacterial peritonitis was made based on clinical findings of generalized abdominal pain, abdominal tenderness, and ascitic fluid showing more than 250 neutrophils/µL.

Data analysis was performed using SPSS version 24. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were calculated for qualitative and quantitative variables such as gender, age, duration of CLD, and the occurrence of culture-negative and culture-positive ascites. Stratification by age, gender, and duration of CLD was performed to control for potential confounders. The Chi-square test was used for post-stratification analysis, with a significance level set at $p \leq 0.05$.

RESULTS

Patient demographics, revealing an average age of 46.17 ± 8.66 years, with the duration of chronic liver disease (CLD) averaging 27.99 ± 16.52 months. Regarding gender distribution, males constitute 78.4% (138) of the sample, while females make up 21.6% (38) (as shown in Table 1).

Table 1

Patient Demographics

Demographics		Mean ± SD / n (%)
Age (years)		46.170±8.66
Duration of Chronic Liver Disease (months)		27.994±16.52
Gender	Male	138 (78.4%)
	Female	38 (21.6%)

Table 2 provides the results of ascites fluid culture, with 50 positive cases (28.4%) and 126 negative cases (71.6%) out of a total of 176 samples.

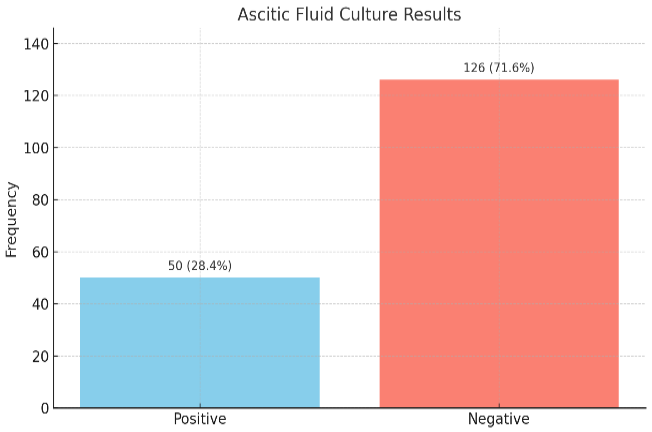
Table 2

Ascites Fluid Culture

Ascites Fluid Culture	Frequency	%age
Positive	50	28.4%
Negative	126	71.6%
Total	176	100%

In Table 3, the association between ascites fluid culture results and demographic factors is analyzed. The age groups of 30-50 years and >50 years show no significant difference in ascites culture positivity (p = 0.892).

Figure 1



However, a significant difference is observed in gender (p = 0.024*), with 32.6% of males showing positive results compared to only 13.2% of females. Duration of CLD also does not significantly affect culture results (p = 0.359).

Table 3

Association of Ascites Fluid Culture with Demographic Factors

Demographic Factors		Ascites Fluid Culture		p-value
		Positive n(%)	Negative n(%)	
Age (years)	30-50	32 (28.1%)	82 (71.9%)	0.892
	>50	18 (29%)	44 (71%)	
Gender	Male	45 (32.6%)	93 (67.4%)	0.024*
	Female	5 (13.2%)	33 (86.8%)	
Duration of CLD (months)	6-12	12 (23.5%)	39 (76.5%)	0.359
	>12	38 (30.4%)	87 (69.6%)	

The logistic regression model presented in Table-IV predicts the likelihood of a positive ascites fluid culture based on age, gender, and duration of CLD. The model is statistically significant (Omnibus Test p = 0.003) and correctly classifies 74.4% of cases. Gender (female) and the duration of CLD significantly impact the likelihood of a positive result. The odds ratio for gender (female) is 3.391 (95% CI: 1.205, 9.545), indicating a higher likelihood of a positive culture among females. The odds ratio for duration of CLD is 0.967 (95% CI: 0.943, 0.992), indicating that a longer duration of CLD decreases the likelihood of a positive result. The Hosmer and Lemeshow test indicates a good model fit (p = 0.013).

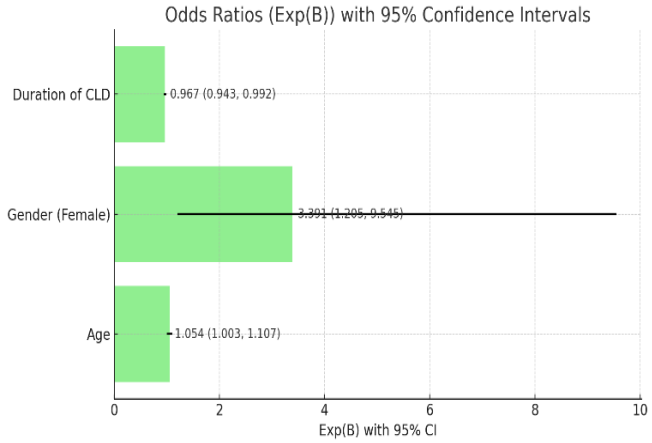
Table 4

Logistic Regression Results for Ascites Fluid Culture Prediction

Variable	B	S.E.	Wald	Sig.	Exp(B)	95% C.I. for Exp(B)
Age	0.052	0.025	4.243	0.039	1.054	(1.003, 1.107)
Gender (Female)	1.221	0.528	5.348	0.021	3.391	(1.205, 9.545)
Duration of CLD	-0.033	0.013	6.792	0.009	0.967	(0.943, 0.992)

Coefficients (B), standard errors (S.E.), Wald statistics, p-values (Sig.), odds ratios (Exp(B)), and 95% confidence intervals for the odds ratios.

Figure 2



DISCUSSION

The observations in this study confirm that a high proportion of spontaneous bacterial peritonitis (SBP) in cases of spontaneous bacterial peritonitis with chronic liver disease (CLD) showed positive cultures (71.6%) and 28.4% with negative cultures, respectively. The finding of a high proportion of positive cultures is in agreement with observations in previous studies and can be supported through factors such as preceding use of antibiotics, the presence of low-virulence and fastidious microorganisms, and technical difficulty in growing specific microorganisms in cultures. The finding of a high proportion of males (32.6%) with positive cultures in comparison with females (13.2%) in the present study can represent a high susceptibility of male cases to SBP and can be considered to represent a high proportion of males with the disease and possibly a variation in immune function and severity of pathology between males and females.

Surprisingly, the disease duration in CLD failed to correlate with cultures in a statistically significant manner. This suggests that the development of SBP in CLD patients is most probably closely linked with acute processes such as bacterial translocation and less with

overall disease duration.

For comparison, studies such as Zaman et al. [19] have documented a prevalence of SBP in cirrhotic patients with ascites, with 56% of cases having SBP, 39.3% with classical SBP, and 57.1% with culture-negative neutrocytic ascites. In agreement with these observations, in our study, a similar proportion of culture-negative ascites (71.6%) was found, but with a larger proportion of cases in our study having negative cultures, in contrast to 57.1% in Zaman et al. One possible variable for such a difference could include differences in demographics in terms of population, with a larger proportion of males in our study, and possibly a larger proportion of males with culture-negative cases.

Ayub et al. [20] have even observed a higher proportion of culture-negative infection in ascitic fluid (77.5%) with a higher proportion of male patients (62.5%). In our case, males have a higher proportion with positive cultures, with 32.6% males having positive cultures. This observation in both studies aligns with a general trend in research that males have a higher proportion with positive cultures in SBP, possibly due to gender-related variation in immune function and/or factors related to comorbidities.

Notably, 52.6% of SBP in Bibi et al. [21] consisted of neutrocytic ascites with no cultures, in contrast with our 71.6% cases with culture-negative ascites. Perhaps a larger proportion in our study, then, could have represented geographical variation in microbial flora, or in laboratory testing protocols. Duration of CLD in our study did not affect cultures ($p = 0.359$), in contrast with observations in studies such as Sajjad et al. [22] who did not specifically assess this variable.

For the logistic regression model, in our analysis, the duration and gender of CLD both played an important role in predicting positive ascites culture. For gender (females), the odds ratio was 3.391, and for the duration of CLD, the odds ratio was 0.967, indicating that both shorter disease duration and female gender were associated with higher odds of a positive culture. These findings agree with Mahato et al. [23] where gender played an important role in predicting the prevalence of SBP. Reduced positivity with increased duration of CLD, however, is a new observation in our analysis. This may suggest that with increased duration, over time, bacterial resistance could develop, leading to less virulent infections that are not detected in cultures, possibly due to altered immune mechanisms in long-standing liver disease.

Our investigation supports and reconfirms findings from current studies regarding the high incidence of culture-negative ascites and the predominance of SBP in male subjects with cirrhotic disease. Furthermore, our observations reaffirm the significant role of gender and duration of cirrhosis in predicting a positive ascitic culture yield. Additionally, gender and disease duration have a notable influence on the development of SBP and warrant further investigation into individualized treatment regimens. Moreover, our investigation highlights the need for continued monitoring of microbial resistance and the potential for a new, region-dependent model for antibiotic therapy, considering the microbial flora in a specific region.

The current study is not without its limitations. To begin with, it is a single-center study, and its generalizability to larger populations could, therefore, be limited. For our analysis, even the sample might not fully represent the clinicopathologic diversity across different regions. Most importantly, a lack of long-term follow-up renders it impossible for us to assess the long-term consequences and recurrence of SBP in such a cohort. Long-term studies with multicenter collaboration and follow-up will be necessary to confirm and extend these observations.

CONCLUSION

Our study reached a conclusion that spontaneous bacterial peritonitis in cirrhotic patients and infection in ascitic fluid with a negative culture is prevalent. In our study, duration and gender have a strong role in deciding positive cultures in ascitic fluid. In conclusion, these factors have to be considered in practice for early intervention and therapy. In our conclusion, continuous observation of microbial resistance and gender-sensitive therapy in treating SBP in such a scenario is significant.

Acknowledgments

The expertise and professionalism of the healthcare team are evident in their meticulous management of patient data. Their ongoing commitment to accurate and thorough documentation fosters effective teamwork, facilitates informed clinical decision-making, and upholds exceptional standards of care quality.

Authors' Contribution

Dr. Muhammad Rizwan Subhani spearheaded the development of the study, handled manuscript preparation, and was responsible for collecting and supervising the hospital data.

Dr. Adnan Salim contributed significantly to refining the study's framework, carried out comprehensive data analysis and interpretation, and offered insightful feedback that strengthened the manuscript's clarity and depth.

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