

Ascitic Fluid High Sensitivity C - reactive protein (Hs-CRP) As a Prognostic Marker in Patients of Cirrhosis with Spontaneous Bacterial Peritonitis (SBP)

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ABSTRACT

Introduction: Cirrhosis and its complications are a major concern for global health with liver transplant being the cure for decompensated cirrhosis. Ascites is the most common complication of cirrhosis with portal hypertension. Early recognition of spontaneous bacterial peritonitis (SBP) has implications on response to treatment and survival, hence necessitates the high degree of clinical suspicion with markers that can guide the prognosis and outcome

Aim: The aim of the study was to establish the role of ascitic fluid high sensitive C-reactive protein (hs -CRP) as a prognostic marker in patients of cirrhosis with SBP.

Material and methods: The study was carried out in the Department of Medicine at Guru Nanak Dev Hospital Of Government Medical College, Amritsar. A total of 50 patients of decompensated cirrhosis were enrolled and were categorized into Groups A and B which included patients with and without spontaneous bacterial peritonitis respectively. Hs-CRP levels of both the groups were estimated. SBP cases were treated with its standard recommended antibiotic therapy and hs-CRP level was again estimated after 5 days of antibiotic therapy or at the time of discharge.

Results: Mean age of people in group A was 48.52 ± 7.94 years whereas in group B was 47.24 ± 6.97 years with males predominance in both groups A and B. Alcoholic Cirrhosis was the most common etiological factor. Ascitic fluid hs-CRP levels on admission were higher in patients of cirrhosis with SBP(Group A) compared to patients of cirrhosis without SBP(Group B). Higher ascitic fluid hs-CRP levels in SBP correlated with poor outcome of the patients.

Conclusion: Higher ascitic fluid hs-CRP levels correlated with poor outcome of the patient making it a valuable prognostic marker in patients of cirrhosis with spontaneous bacterial peritonitis.

Keywords: Cirrhosis, Ascites, hs-CRP, Spontaneous bacterial peritonitis

INTRODUCTION

Cirrhosis is a chronic progressive disease of liver characterized by extensive degeneration and destruction of the liver parenchymal cells and in an attempt to regenerate, normal liver architecture is replaced by abnormal nodules that lack normal lobular organization. The patient's clinical history combined with serologic and histologic evaluation identifies the etiology of cirrhosis¹. Ascites is the most common complication of cirrhosis with portal hypertension. It is the accumulation of excess fluid

within the peritoneal cavity. The development of ascites correlates with a poor prognosis, with an increase in mortality to 15% at one-year and 44% at five-year follow-up respectively^{2,3}.

Spontaneous bacterial peritonitis (SBP) is a common and severe complication of ascites characterized by spontaneous infection of the ascitic fluid without an intraabdominal source of infection.⁴ Patients with cirrhosis who are in a decompensated state are at the highest risk of developing spontaneous bacterial

peritonitis⁵ and is the major cause of mortality and morbidity among them.

Tillet and Francis discovered CRP in 1930 and they proposed the hepatic synthesis of CRP. It is a positive acute phase protein found in the blood stream, the levels of which rises in response to inflammation. CRP is synthesized predominantly in hepatocytes but is also produced by endothelial cells, macrophages, smooth muscle cells, lymphocytes, and adipocytes.

Role of CRP as a marker of inflammation has been extensively studied in Coronary Artery Diseases (CAD) and in other conditions like chronic liver diseases, collagen vascular diseases and inflammatory arthritis. CRP has also been studied as an inflammatory marker in alcoholic liver disease, infective hepatitis, autoimmune hepatitis, cirrhosis and SBP. Studies have supported the view that CRP levels increases in decompensated cirrhosis and infections in cirrhosis⁶⁻⁸ Induction of C-reactive protein in hepatocytes is principally regulated at the transcriptional level by the cytokine interleukin-6 (IL-6), an effect which can be enhanced by interleukin-1 β (IL-1 β).⁹

In recent years with development of highly sensitive assays, inflammatory biomarkers such as procalcitonin (PCT) and C-reactive protein (CRP) have been vigorously investigated as tools for early diagnosis of bacterial infection in a variety of clinical settings.^{10,11} CRP is a sensitive but nonspecific systemic marker of inflammation. It also rises dramatically in other situations including trauma, burns, myocardial infarction and cancer i.e. potential reasons for admission to intensive care.

Nevertheless, some studies have shown that CRP is a reliable marker for the diagnosis of bacterial infection in patients with chronic liver disease.^{12,13} With recent advances in technology, CRP values can be measured at very low levels with highly sensitive assays called high sensitive CRP(hs-CRP). However, there is controversy among studies regarding the clinically significant cut-off levels of hs-CRP that differentiates infected from non-infected cirrhotic patients. Hence, if these findings could be incorporated into clinical practice, empirical antibiotics could be administered in patients with high values but borderline documentation of infection and stopped in those with low values and no definitive evidence of infection.

Hence this study was carried out to assess the correlation of values of ascitic fluid high sensitivity C-reactive protein levels in patients of cirrhosis with and without spontaneous bacterial peritonitis and the prognostic significance of ascitic fluid high sensitive c-reactive protein levels in patients with spontaneous bacterial peritonitis who receive appropriate antibiotic therapy.

MATERIAL AND METHODS:

The present prospective study was carried out in the Department of Medicine at Guru Nanak Dev Hospital Of Government Medical College, Amritsar. The study was initiated only after obtaining permission from the Institutional Ethics Committee, Guru Nanak Dev Hospital, Amritsar. The patients were explained about the procedures and an informed consent was taken prior to enrolment in the study.

A total sample of 50 patients of decompensated cirrhosis were included in the study and divided into Group A and Group B. The groups were categorized as:

- 1) Group A comprised of 25 patients of decompensated cirrhosis with spontaneous bacterial peritonitis aged 18 years and above.
- 2) Group B comprised of control group with 25 patients of decompensated cirrhosis without spontaneous bacterial peritonitis aged 18 years and above were included.

EXCLUSION CRITERIA :

Coronary artery disease

Collagen vascular disorders

Any form of inflammatory joint disorders

Any intra abdominal source of infection other than spontaneous bacterial peritonitis

Auto immune hepatitis

Growths of more than one organism in ascitic fluid culture were excluded from the study.

DEFINITION OF CASES AND CONTROLS:

The definitive diagnosis of spontaneous bacterial peritonitis was made on the basis of ascitic fluid examination.

- 1) Patients with a positive monomicrobial ascitic fluid bacterial culture and an elevated

ascitic fluid absolute polymorpho nuclear leucocyte (PMN) cell count of ≥ 250 cells/mm, in absence of any intra abdominal source of infection.

- 2) Patients with Culture Negative Neutrocytic Ascites (CNNA) with ascitic fluid absolute polymorpho nuclear leucocyte (PMN) cell count ≥ 250 cells/mm³ without any intra abdominal source of infection were also considered as spontaneous bacterial peritonitis.

Chronic liver disease was classified on the basis of Child–Pugh score. The criteria for decompensated cirrhosis was presence of cirrhosis of liver sonographically along with any of the features of decompensation manifesting as presence of portal hypertension in the form of ascites or variceal bleed or hepatic encephalopathy or Patients classified as Child Pugh score class B and above.

Ascitic fluid was obtained by diagnostic abdominal paracentesis under sterile precautions and ascitic fluid complete examination (appearance, colour, total ascitic fluid protein, ascitic fluid albumin, ascitic fluid total cell count, ascitic fluid differential cell count) and ascitic fluid high sensitive C-reactive protein levels were estimated at the time of admission in both study group A and group B.

Spontaneous bacterial peritonitis cases (Group A) were treated with standard recommended antibiotic therapy (inj. cefotaxime 2 g 12 hourly for 5 days). Ascitic fluid high sensitive C reactive protein levels

were again estimated after 5th day of antibiotic therapy or at the time of discharge.

Laboratory investigations included complete blood count, Liver function tests, Renal function tests, ultrasound whole abdomen, prothrombin time & international normalized ratio, urine complete examination, stool for occult blood, ascitic fluid complete examination, ascitic fluid culture and ascitic fluid quantitative high sensitive c-reactive protein.

STATISTICAL ANALYSIS:

Data collected was statistically analysed with help of Epi Info (TM) 3.5.3 which is a trademark of the Centers for Disease Control and Prevention (CDC). Test of proportion (Z-test) was used to test the significant difference between two proportions. t-test was used to test the significant difference between means. Odds ratio (OR) with 95% Confidence Interval (CI) was calculated to measure the different risk factor. Significance level was set at 0.05 ($p < 0.05$) and confidence intervals were at 95% level.

RESULTS:

The mean age of people in group A was 48.52 ± 7.94 years whereas in group B was 47.24 ± 6.97 years. The number of males were higher than females in both groups A and B. Alcohol was the most common etiology for cirrhosis in both the groups with 19 (76%) patients in Group A and 17 (68%) patients in Group B.(Table 1)

Table 1 : Age, Gender and Etiological distribution in Group A and Group B

| Parameter | Group A | Group B | P value |
|-----------------------------|------------------|------------------|---------|
| Age(years) Mean \pm SD | 48.52 \pm 7.94 | 47.24 \pm 6.97 | - |
| Male(%) | 23(92%) | 22(88%) | 0.637 |
| Female(%) | 2(8%) | 3(12%) | |
| Alcoholic(%) | 19(76%) | 17(68%) | 0.52 |
| HBV(%) | 3(12%) | 4(16%) | 0.68 |
| HCV(%) | 3(12%) | 4(16%) | 0.68 |

Baseline investigations of both Group A and Group B are summarised in table 2

Table 2: Baseline laboratory investigations of Group A and Group B

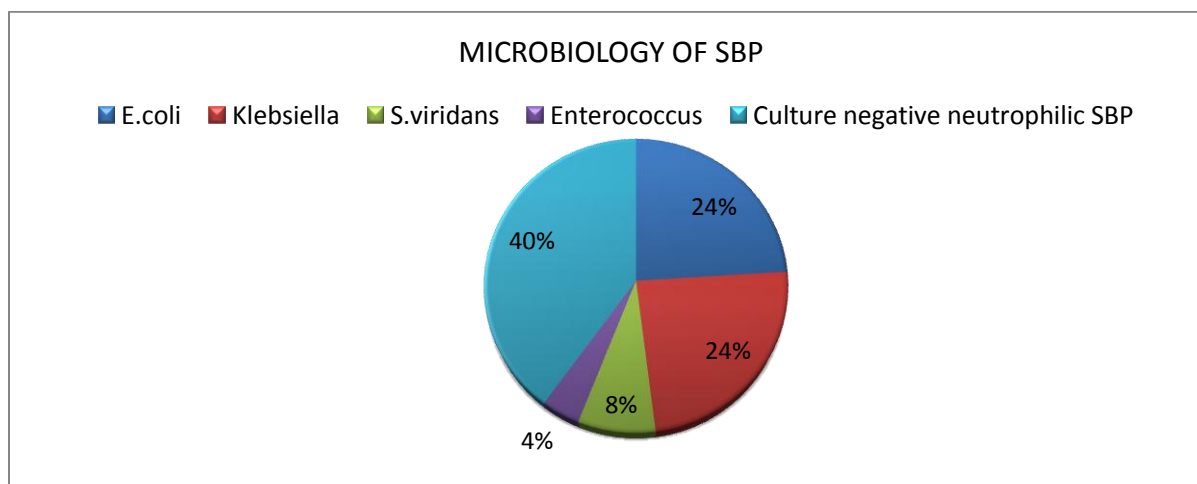
| | GROUP A (CASE) | | GROUP B (CONTROL) | | P VALUE |
|--|-------------------|-------|----------------------|-------|------------|
| | MEAN | SD | MEAN | SD | |
| SERUM BILIRUBIN (mg/dl) | 3.92 | 1.73 | 2.15 | 1.08 | 0.0001* |
| SERUM ASPARTATE AMINOTRANSFERASE(U/L) | 97.44 | 34.24 | 84.64 | 22.62 | 0.124 |
| SERUM ALANINE AMINOTRANSFERASE (U/L) | 62.8 | 21.0 | 57.84 | 16.29 | 0.355 |
| SERUM ALKALINE PHOSPHATASE (U/L) | 76.8 | 11.28 | 74.44 | 18.35 | 0.58 |
| SERUM ALBUMIN (gm/dl) | 3.36 | 0.408 | 3.44 | 0.345 | 0.345 |
| SERUM CREATININE (mg/dl) | 1.52 | 0.559 | 1.08 | 0.48 | 0.004 |

The number of patients belonging to Child-Pugh Class-A were 6 (24%) in Group A and 10(40%) in Group B. The number of patients belonging to Child-Pugh Class-B were 8 (32%) in Group A and 8(32%) in Group B. The number of patients belonging to Child-Pugh Class-C were 11 (44%) in Group A and 7(28%) in Group B. The difference between Group A and Group B was statistically insignificant($p>0.05$).(Table 3)

Table 3: Distribution of patient's according to child pugh score in Group A and Group B.

| CHILD PUGH SCORE | GROUP A | | GROUP B | | P value |
|---------------------|---------|-----|---------|-----|---------|
| | N | % | N | % | |
| CLASS A | 6 | 24% | 10 | 40% | 0.38 |
| CLASS B | 8 | 32% | 8 | 32% | |
| CLASS C | 11 | 44% | 7 | 28% | |
| TOTAL | 25 | 100 | 25 | 100 | |

The culture results of ascitic fluid in patients of spontaneous bacterial peritonitis showed that culture negative neutrophilic spontaneous bacterial peritonitis was the predominant form (40%) followed by E.coli (24%), Klebsiella(24%), S.viridans (8%), and Enterococci(4%).(Figure1)

Figure 1: Culture results of ascitic fluid in patients of spontaneous bacterial peritonitis (GroupA)

The mean value of ascitic fluid hs-CRP levels in patients of Group A before antibiotic therapy was 69.04 ± 18.46 mg/dl. The mean value of ascitic fluid hs-CRP levels in patients of Group A after antibiotic therapy was 46.56 ± 15.15 mg/dl. The difference in mean values of ascitic fluid hs-CRP levels in patients of spontaneous bacterial peritonitis (Group A) before and after antibiotic therapy was statistically significant ($p=0.0001$). (Table 5)

The mean value of ascitic fluid total neutrophil counts in patients of Group A before antibiotic therapy was 392 ± 83.75 cells/cu.mm. The mean value of ascitic fluid total neutrophil counts in patients of Group A after antibiotic therapy was 331 ± 68.19 cells/cu.mm. The difference in mean values of ascitic fluid total neutrophil count in patients of spontaneous bacterial peritonitis (Group A) before and after antibiotic therapy was statistically significant ($p=0.006$). (Table 4)

Table 4: Mean ascitic fluid hs-crp levels and mean ascitic fluid total neutrophil counts in Group A patients before and after antibiotic treatment.

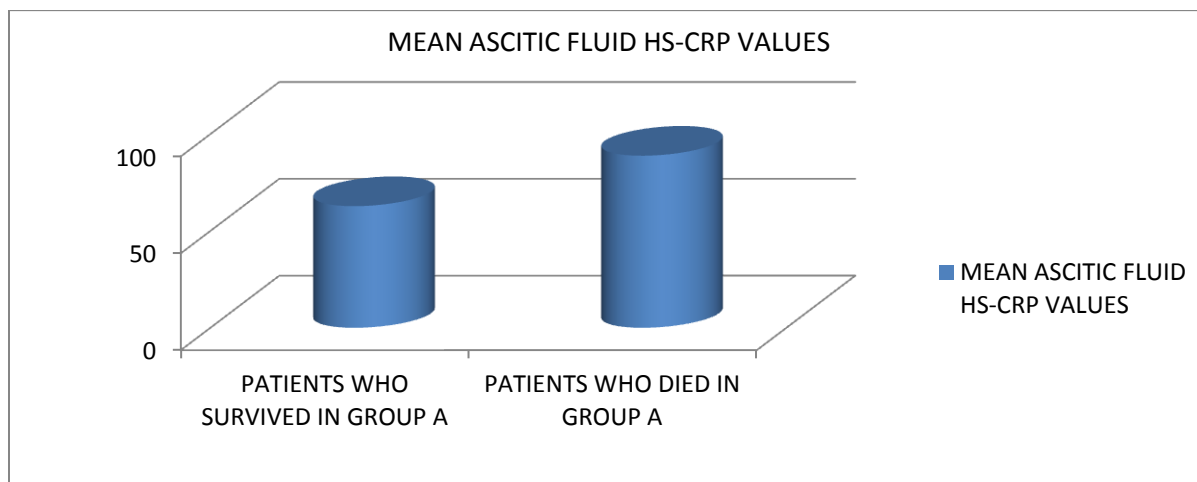
| | Group A at admission (Before antibiotic treatment) | | Group A (After antibiotic treatment at 5th day) | | P value |
|--|---|-------|--|-------|---------|
| | MEAN | SD | MEAN | SD | |
| MEAN ASCITIC hs-CRP LEVELS (mg/dl) | 69.04 | 18.46 | 46.56 | 15.15 | 0.0001* |
| ASCITIC FLUID TOTAL NEUTROPHIL COUNT (Cells/cu.mm) | 392 | 83.75 | 331 | 68.19 | 0.006* |

Death was final outcome in 6 patients (24%) in group A and in 1 patient (4%) in group B. The mean value of ascitic fluid hs-CRP levels of patients who survived in Group A was found to be 62.78 ± 16.47 mg/dl and the mean value of ascitic fluid hs-CRP levels of patients who died in Group A was found to be 88.83 ± 6.33 mg/dl. This difference in mean values of ascitic fluid hs-CRP levels was found to be statistically significant ($p < 0.001$). (Table 5/Figure 2)

Table 5: Comparison of ascitic fluid hs-crp values between patients with respect to outcome in Group A

| | PATIENTS WHO SURVIVED IN GROUP A (N=19) | PATIENTS WHO DIED IN GROUP A (N=6) | P VALUE |
|------------------------------------|---|--|------------|
| MEAN ASCITIC FLUID hsCRP LEVELS | 62.78 ± 16.47 | 88.83 ± 6.33 | $<0.001^*$ |

Figure 2: Comparison of ascitic fluid hs-crp values between patients with respect to outcome in Group A



DISCUSSION:

The present study had a maximum number of patients in the age group of 41-50 for both groups A and B. Gender distribution revealed that males outnumbered the females in both Group A and Group B. This was in similarity to the findings of the study done by Kadam et al¹⁴.

Liver cirrhosis can be caused by varied etiologies. In this study alcohol was the most common etiology for cirrhosis in both the groups. This comes in harmony with the highest incidence and prevalence of alcoholic cirrhosis in India with respect to literature making it the most common cause of cirrhosis among Indian patients.

We observed that the difference in mean serum bilirubin values between patients in Group A and Group B was found to be statistically significant

($p < 0.05$) which was in accordance with studies conducted by Papp et al¹⁵ and Kadam et al¹⁴. With statistically significant difference in mean serum bilirubin values between SBP and without SBP group it is reinforced that SBP is one of the important causes for sepsis induced acute insult on chronic liver disease in SBP patients and thereby increased mortality.

Further even the difference in mean serum creatinine values between patients with SBP and without SBP was statistically significant and it is in harmony with the studies conducted by Papp et al¹⁵ and Kadam et al¹⁴. This emphasizes the increased incidence of hepato-renal syndrome in patients of decompensated cirrhosis with SBP.

In the present study, 76 % of patients with SBP were in Child Pughs class C or B and 60 % of patients

without SBP were in Child Pughs class C or B. The difference is statistically insignificant between patients with SBP and patients without SBP. This is in contrast to the observations made by Kadam et al¹⁴ where 78% of patients with SBP were Child Pugh class B or C and 58% of patients without SBP were Child Pugh Class B or C and the difference was statistically significant($p<0.05$).

It was observed that culture negative neutrophilic spontaneous bacterial peritonitis was the most predominant form in the present study accounting for 40 of the patients with SBP. It was in contrast to the observation made by Sara et al¹⁶, where culture positive spontaneous bacterial peritonitis accounted for 65% of the cases with the most common isolated organism being E.coli accounting for 33.8% of the SBP patients. This difference may be attributed to the methodology behind the culture of ascitic fluid.

We observed that the mean values of ascitic fluid total neutrophil counts before and after antibiotic therapy was statistically significant ($p=0.006$). This was consistent with the observations made by B.A.Runyon¹⁷ where they observed the mean ascitic fluid polymorphonuclear counts of patients with SBP on Day 1 to be 6534 ± 6181 cells/cu.mm and there was a 92% reduction in mean ascitic PMN counts after two days of appropriate antibiotic therapy and the difference in mean values before and after antibiotic therapy statistically significant($p<0.05$).

Preto-Zamperliniet al¹⁸ reported a significant elevation of CRP in serum in patients with SBP, leading to the inference that CRP was an independent variable in the prediction of SBP. Also, in study by Yildirimet al¹⁹, it was found that CRP was increased in the serum of SBP patients. But in another study done by B.A.Runyon et al¹⁷ they inferred that Ascitic fluid CRP did not appear to be a useful indicator of ascitic fluid infection; This may be due to the use of laser nephelometry in measuring the level of CRP (less accurate than hs-CRP).

Accordingly in our study we observed that the difference in the mean ascitic fluid hs-CRP values of group with SBP and without SBP was statistically significant. Our results came in agreement with Kadam et al¹⁴ who found that the mean level of ascitic fluid hs-CRP before antibiotic therapy of the patients with SBP was 77.20 ± 11.65 mg/dl and the mean level of hs-CRP of the cirrhotic patients

without spontaneous bacterial peritonitis was 42.50 ± 7.48 mg/dl.

In present study, the mean values of ascitic fluid hs-CRP in Group A before antibiotic therapy was 69.04 ± 18.46 and 5 days after antibiotic therapy was 46.56 ± 15.15 mg/dl. The difference in mean values of ascitic fluid hs-CRP before and after antibiotic therapy was statistically significant($p<0.05$). This was in agreement with the observations of Mohammed et al²⁰ where the mean ascitic fluid hs-CRP levels before and after antibiotic treatment showed a reduction of mean ascitic fluid hs-CRP levels by 69% and was statistically significant.

In present study, the number of patients who died in Group A was 6(24%) and in Group B was 1(4%). It was in accordance with various studies^{14,18} where mortality was more in SBP group compared to patients without SBP. Again it was noticed that the patients who died in Group A were found to have higher levels of baseline ascitic fluid hs-CRP levels with the mean values being 88.83 ± 6.33 mg/dl compared to 62.78 ± 16.47 mg/dl in patients who survived in Group A. This difference was statistically significant ($p<0.001$). This was in agreement with observation of Kadam et al¹⁴ where the difference in mean ascitic fluid hs-CRP values between patients who died and patients who survived in SBP group was statistically significant($p<0.05$).

CONCLUSION:

Hence, the present study concluded that higher ascitic fluid hs-CRP level correlates with poor outcome in patients of cirrhosis with spontaneous bacterial peritonitis making it a valuable prognostic marker.

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