Role of ascitic fluid C3 in spontaneous bacterial peritonitis

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Abstract  Background: The C3 component of complement tends to be reduced in cirrhosis and patients with reduced ascitic fluid C3 concentration and reduced opsonic activities have been shown to be predisposed to SBP [1].

Aim of the work: To compare the level of ascitic fluid C3 concentration in cirrhotic patients with and without spontaneous bacterial peritonitis to determine its possible protective role against acquiring infection.

Methods: This is a prospective case-control study in which we recruited 45 cirrhotic patients presenting with ascites, of which 25 showed evidence of SBP. All patients had diagnostic paracentesis, received the appropriate treatment, discharged and followed-up monthly for 3 months, with ascitic fluid C3 measurement. Ascitic fluid C3 was compared between both groups at baseline and for three successive reading over 3 months. It was also compared in the same patients group over this interval and correlated with other AF parameters at baseline reading.

Conclusion: Ascitic fluid C3 is reduced in cirrhotic patients with SBP and stays lower compared with those without the infection after the first episode. So we conclude that C3 plays an important role in the local defense of ascitic fluid and needs further long term follow-up studies to evaluate its role as a predictor of prognosis for cirrhotic ascitic patients.

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1. Introduction

Ascites is the most common complication in patients with decompensated cirrhosis. Approximately 50% of patients with compensated cirrhosis will develop ascites over a 10-year period [2]. Patients with cirrhosis and ascites show higher susceptibility to bacterial infections mainly because of the inadequate defense mechanisms. The most frequent and most severe one being spontaneous bacterial peritonitis (SBP). Prevalence of Spontaneous bacterial peritonitis in cirrhotic patients with ascites is as high as 18%, with 40–70% associated mortality [3]. Spontaneous bacterial peritonitis is probably related to several impaired defense mechanisms, such as depressed reticuloendothelial system phagocytic activity,
leukocyte dysfunction, reduced serum complement, and low bactericidal activity of ascitic fluid [4]. Ascitic fluid complement (C3) concentration and opsonic activities are important protective factors against SBP. The C3 component of complement tends to be reduced in cirrhosis and patients with reduced ascitic fluid C3 concentration and reduced opsonic activities have been shown to be predisposed to SBP [1].

This study was designed with the aim to compare the level of ascitic fluid C3 concentration in cirrhotic patients with and without spontaneous bacterial peritonitis (SBP) monthly over a period of 3 months, to determine its possible protective role against this infection.

2. Patients and methods

This is a prospective case-control study, conducted in the internal medical department and the outpatient clinics of Ain Shams University Hospitals, from October 2009 till October 2010. A total of 45 patients were recruited for the study; 25 cirrhotic patients admitted with the diagnosis of spontaneous bacterial peritonitis (group I) and 20 patients with the diagnosis of cirrhotic ascites without evidence of peritonitis as controls (group II). The diagnosis of SBP was based on the presence of ascitic fluid (AF) polymorphonuclear (PMN) cell count of 250 cells/ml or more, in the absence of secondary peritonitis, with or without positive ascitic fluid culture.

2.1. Exclusion criteria

Patients who had received antibiotics or diuretics up to one week prior to admission were excluded. Other exclusion criteria were hepatocellular carcinoma, portal vein thrombosis, hepatorenal syndrome and diabetes mellitus. We ruled out patients with the history of recent antibiotic use, to ensure matched groups of patients and controls. The rest of exclusion criteria were mainly concerned with their potential effect on protein synthesis and loss.

At enrollment, complete physical examination was done including signs of decompensated liver cirrhosis mainly the presence of ascites. Basic laboratory work up including: liver function test, renal function test, fasting and postprandial blood glucose, complete blood count, prothrombin concentration and INR were performed for all patients. In addition, testing for the presence of hepatitis B surface antigen (HBs Ag), hepatitis B core antibody (HBe Ab) and hepatitis C virus antibody (HCV Ab) was done for all patients.

Diagnostic paracentesis was performed for all patients on admission under strict aseptic conditions under ultrasonographic guidance. The ascitic fluid (AF) obtained was tested for: glucose, total proteins, albumin, LDH and polymorphonuclear leukocytic count (PMNLs). Ascitic fluid cultures were performed by inoculating 10 ml of the fluid in aerobic and anaerobic blood culture bottles at bedside and incubated for 48 h at 37 °C. Complement 3 (C3) determinations in AF were measured by nephelometry (Beckman ARRAY™ Beckman instrument Co., California) using anti-C3 antiserum.

Patients in both groups were given the appropriate treatment during their hospital stay. Patients with SBP were given antibiotics immediately after taking ascitic fluid samples. A combination of third generation cephalosporin and metronidazol intravenously was the standard treatment. Average hospital stay was 7–10 days.

Discharged patients in both groups were followed-up monthly for three successive months for repeat clinical examination in the outpatient clinics. Ascitic fluid samples were collected each visit for the re-measurement of C3 level.

By the end of the study, the total number of patients completing three successive follow-up visits in the outpatient clinic was 15 in each group. Drop out was either due to death or failure to show up for the repeat examinations.

This study was approved by the local ethics committee of the Ain Shams University, and an informed written consent was obtained from all patients before enrollment in this study.

2.2. Statistical analysis

The statistical analysis was performed using the SPSS (Statistical Program for Social Sciences), version 13. Data are expressed as means ± SD. Comparisons between groups were performed using the Student’s t-test for quantitative variables and Chi square for qualitative variables. Comparison of ascitic fluid C3 levels across the study period was performed for each group separately using repeated measures ANOVA. Comparison between the various pairwise combinations of baseline and follow-up readings of ascitic fluid C3 levels was performed using the paired t-test for each group separately. Correlations were performed using the Pearson’s correlation coefficient. A P-value of less than 0.05 was considered significant.

3. Results

Demographic criteria: Group I included 11 males (73.33%) and four females (26.67%) while group II included 10 males (66.67%) and five females (33.33%). Ages ranged from 44 to 58 with a mean of 51 ± 4.66 years in group I and from 41 to 57 with a mean of 50.4 ± 4.306 years in group II. There was no statistically significant difference as regards both age and sex distribution between both the study groups (P = 0.717 and 0.69, respectively).

Child-Pugh class: Four patients were Child-Pugh class B (26.67%) and 11 patients were Child-Pugh class C (33.33%) in group I, while 10 patients were Child-Pugh class B (66.67%) and five patients were Child-Pugh C (33.33%) in group II. There was a statistically significant difference between both groups as patients of group I showed more advanced Child-Pugh class (P = 0.028).

Etiology of liver cirrhosis: Group I included nine HCV positive (60%), four HBV positive (26.66%) and two combined HCV and HBV positive (13.33%) patients. Group II included 12 HCV positive (80%), three HBV positive (20%) patients. There was no statistically significant difference as regards etiology of cirrhosis between both groups (P = 0.276).

Ascitic fluid culture. Ascitic fluid cultures were positive in 10 patients (66.67%) (classic SBP) and negative in five patients (33.33%), i.e. culture negative neutrocytic ascites (CNNA) in group I. Ascitic fluid cultures were negative in all patients of group II. Among the 10 positive cultures, eight (i.e. 80%) were caused by Gram-negative enterobacteriaceae (five Escherichia coli and three Klebsiella pneumoniae) and two (i.e. 20%) were caused by Gram-positive cocci (one Staphylococcus aureus and one Streptococcus pneumoniae).
### 3.1. Laboratory results

As shown in Table 1, comparing both groups as regards complete blood count, liver and kidney function tests showed significantly higher serum total leukocytic count and lower serum albumin level in group I (\(P = 0.000\) and 0.005, respectively), while the rest of studied variables did not show any statistical difference between both the study groups.

**Ascitic fluid examination:** Ascitic fluid neutrophil count and LDH level was significantly higher in patients with SBP compared to those in group II (\(P\)-value 0.000 and 0.003, respectively), while total proteins, albumin and glucose levels were significantly lower in the patient of group I (\(P\)-value 0.000, 0.028 and 0.021, respectively) as shown in Table 2.

### 3.2. Complement 3 levels in ascitic fluid

Comparing C3 levels between both groups: Ascitic fluid complement 3 levels were significantly lower in patients with SBP (group I) than ascitic patients without SBP (group II) both at baseline measurement and the follow-up readings as shown in Table 3 and Fig. 1.

Comparing C3 levels during follow-up in each group: Table 4 shows a significant reduction in ascitic fluid complement 3 level in group I, over the follow-up period of 3 months compared with baseline reading on admission (\(P = 0.026\)), while this was not observed in group II, patients who had no evidence of SBP (\(P = 0.099\)).

**Ascitic fluid complement 3 (C3) in different Child-Pugh classes:** When compared to Child-Pugh class “B” patients, C3 levels were significantly lower in Child-Pugh class “C” patients in both the study groups independent of SBP presence, Table 5.

**Ascitic fluid C3 correlation with other AF variables:** Ascitic fluid C3 showed significant positive correlation with AF total protein content (\(P = 0.000\)) while it showed significant negative correlation with the fluid neutrophil count and LDH level (\(P = 0.000\) and 0.011, respectively), as shown in Table 6.

### 4. Discussion

SBP is bacterial infection of the ascitic fluid without any intra-abdominal source of infection [5]. We found that age and sex have no effect on the incidence of SBP as there was no statistically significant difference as regards both variables between groups I and II. This was previously reported more than a decade ago by Puri et al. [6]. Advanced Child-Pugh class on the other hand was significantly higher in group I patients (with SBP) meaning that the severity of the liver disease is probably an important risk factor for the development of SBP [3].

Etiology of liver cirrhosis was not significantly different between both our study groups, with HCV infection being the most frequent cause of chronic liver disease, as shown before by Abaza et al. [7]. The causative organism was isolated in
ascitic fluid culture in about two-thirds of our group I patients, while about a third of them represented culture negative neutrocytic ascites. *E. coli* and *Klebsiela* were the most frequently detected organisms in AF cultures, while Gram-positive cocci were detected in 20% of the cultures. This is in agreement with Park et al., who found that Gram-negative organisms account for more than 60% of the cases of SBP [8].

Comparing both groups as regards complete blood count, liver and kidney function tests showed significantly higher serum total leukocytic count and lower serum albumin level in group I. Peripheral leukocytosis was repeatedly reported with SBP as well as hypoalbuminemia as a marker of liver dysfunction [9]. The rest of liver and kidney functions were not significantly different between both the groups. Although S. bilirubin and prothrombin times are both determining factors in the Child-Pugh class of our patients, yet they were not significantly affected in group I compared to group II. This might be explained by the additive important role for albumin in fighting infections in the ascitic fluid [10].

Ascitic fluid examination showed significantly higher neutrophil count as well as LDH level, while total proteins, albumin and glucose levels were significantly lower in patients of group I compared with group II. High ascitic fluid neutrophil count has been considered as the corner stone for the diagnosis of SBP [11]. Consequently, the high LDH level in infected ascitic fluid can be explained by either diffusion from the blood or by the release from disintegrating AF neutrophils [12].

<table>
<thead>
<tr>
<th>Group</th>
<th>AF complement (C3) (mg/dL)</th>
<th>T-test</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7.157 ± 1.272</td>
<td>7.595</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5.8–9.7</td>
<td>6.401 ± 0.653</td>
<td>9.639</td>
<td>0.001</td>
</tr>
<tr>
<td>Group II</td>
<td>8.7–14.2</td>
<td>11.498 ± 1.941</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>6.472 ± 0.726</td>
<td>8.617</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>5.8–8.2</td>
<td>6.607 ± 0.880</td>
<td>8.079</td>
<td>0.001</td>
</tr>
<tr>
<td>Group II</td>
<td>7.9–14.0</td>
<td>11.320 ± 2.081</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1** The persistently lower level of C3 in group I compared to group II.

**Table 4** Comparison of ascitic fluid C3 in each group across the study period.

<table>
<thead>
<tr>
<th>SBP group</th>
<th>AF C3 (mg/dL), Mean ± SD</th>
<th>Non-SBP group</th>
<th>AF C3 (mg/dL), Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7.157 ± 1.272</td>
<td>0.026</td>
<td>Baseline</td>
<td>11.685 ± 1.928</td>
</tr>
<tr>
<td>1 month</td>
<td>6.401 ± 0.653</td>
<td>1 month</td>
<td>11.498 ± 1.941</td>
<td></td>
</tr>
<tr>
<td>2 months</td>
<td>6.472 ± 0.726</td>
<td>2 months</td>
<td>11.363 ± 2.075</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>6.607 ± 0.88</td>
<td>3 months</td>
<td>11.320 ± 2.081</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5** AF complement 3 levels in Child-Pugh classes B and C patients in groups I and II.

<table>
<thead>
<tr>
<th>Child-Pugh class</th>
<th>AF complement (C3)</th>
<th>T-test</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>8.2–9.7</td>
<td>6.602 ± 0.920</td>
<td>3.872</td>
<td>0.002</td>
</tr>
<tr>
<td>Group II</td>
<td>5.8–8.6</td>
<td>11.685 ± 1.928</td>
<td>4.074</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Table 6** AF complement 3 correlation with AF variables.

<table>
<thead>
<tr>
<th>AF complement (C3)</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF neutrophil count</td>
<td>−0.719</td>
<td>0.000</td>
</tr>
<tr>
<td>AF total protein</td>
<td>0.705</td>
<td>0.000</td>
</tr>
<tr>
<td>AF albumin</td>
<td>0.289</td>
<td>0.121</td>
</tr>
<tr>
<td>AF LDH</td>
<td>−0.459</td>
<td>0.011</td>
</tr>
<tr>
<td>AF glucose</td>
<td>0.284</td>
<td>0.128</td>
</tr>
</tbody>
</table>
the other hand, low protein and albumin concentration in the ascitic fluid have been identified as risk factors for SBP [13], while ascitic fluid glucose could be consumed by bacteria during uncontrolled infection [14].

In both groups the mean ascitic fluid C3 concentration was significantly lower in patients of Child-Pugh class C than in those of Child-Pugh class B, which can be explained by changes in hepatic synthesis pathway [4]. The presumed mechanism for the decrease of C3 in the ascitic fluid is a combination of dilution, low hepatic synthesis, and greater consumption of C3 due to an activation of the alternative complement pathway [4]. Runyon reported that ascitic fluid protein concentration less than or equal to 1.0 g/dl, was associated with a significantly higher risk for spontaneous peritonitis. Our SBP patients had ascitic fluid protein of 1.1 g/dl; which, though significantly lower than in patients without peritonitis, were not to be considered high risk based on AF protein alone. This may highlight the need for an additional marker for the risk of SBP. Complement 3 is an important protein which offers local defense against bacterial infections in ascitic fluid [15]. This can explain its significantly reduced level in group I patients (with the SBP) in our study groups. The finding that ascitic fluid C3 level remained lower in group I compared with group II over a follow-up period of 3 months, supports our assumption that C3 reduction may be considered as a factor in the occurrence of infection, rather than being consumed during the infection. Moreover, there was further significant reduction of the already low level of ascitic fluid C3 in group I compared with same patients’ baseline reading. This further reduction was not noticed in group II patients who did not have SBP. This suggests the negative impact this infection has on liver functions, leading to further deterioration, rather than the natural course of the cirrhosis. These findings confirm that SBP is a recurrent problem and that continuous antibiotic prophylaxis is a safe practice.

C3 level was significantly negatively correlated with AF neutrophil count and LDH which confirms its value as a negative diagnostic marker of the infection. On the other hand, C3 was significantly positively correlated with ascitic fluid protein, as shown in our results in baseline measurements, which explains the reduction in the ascitic fluid opsonic activity [16] predisposing to the occurrence of SBP and confirming its predicted role in the local defense against bacterial infection of ascitic fluid. It can also be considered a potential predictive marker of SBP, added to AF protein level, to enhance the accuracy of the AF analysis.

In conclusion, our study confirms the role of ascitic fluid C3 in the local defense against bacterial infection of ascitic fluid in cirrhotic patients. We also suggest that the reduced level of AF complement 3 levels, in patients with SBP are more likely to be a predisposing factor rather than consumption by alternate complement activation due to the infection. The persistently lower level of AF C3 and its further reduction over time in patients, who had one episode of SBP, should be further evaluated to be used as a predictive and prognostic marker increasing the sensitivity and specificity of ascitic fluid analysis.

Disclaimer

To the best of our knowledge, there is no conflict of interests as regards our authors, nor there any financial aid from any institute, to announce.

References