Significance of C3 in Egyptian Cirrhotic Patients with Ascites Complicated By Spontaneous Bacterial Peritonitis

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Abstract: In cirrhotic patients, the bactericidal and opsonic activity of the ascitic fluid is lower than that observed in non cirrhotic ascites. **Aim of the work:** to assess the association between serum and ascitic fluid levels of C3 in patient with cirrhotic ascites complicated by spontaneous bacterial peritonitis (SBP). **Patients and Methods**:study was conducted on 50 subjects,Group I: **(**20) patients with cirrhotic ascites complicated by SBP,Group II:**(**20) patients with cirrhotic ascites without SBP**,** Group III: (10) patients with non cirrhotic ascites. All patients were subjected to clinicolaboratory investigations,serum,ascetic C3, examination of ascitic fluid and abdominal ultrasonography. **Results:** Patients with cirrhotic ascites complicated by(SBP)had significantly low levels of ascitic and serum C3 compared to those without SBP and with non cirrhotic ascites. There was a statistically significant difference in serum C3 level between Child's B and Child's C.There was a positive correlation between serum C3 with ascitic C3 and albumin. **Conclusion**: The deficiency of serum complement concentrations of C3 is influenced with more progress of the disease and presence of ascites.In patients with cirrhosis and ascites, follow up of complement concentrations may help to recognize patients with increased risk for the development of SBP. Serum C3 of 45g/L and ascitic C3 of 11g/L was the best cutoff value for discriminating patients with SBP from patients without SBP.

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Key words: Liver cirrhosis; Spontaneous bacterial peritonitis; Serum C3; Ascitic C3.

Introduction:

Spontaneous bacterial peritonitis (SBP) is defined as the infected ascitic fluid in absence of any recognizable primary cause of peritonitis **[1].**It develops in 10-30% of hospitalized patients and the mortality exceeded 90%, when it was first described. However, with the early recognition of disease and prompt and appropriate antibiotic treatment, the in-hospital mortality of an episode of SBP has been reduced to approximately 20% **[2].**

Spontaneous bacterial peritonitis is diagnosed when the ascitic fluid culture grows pathogenic bacteria, the ascitic fluid neutrophils count ≥ 250 cells /mm3 and there is no evidence of surgically treatable intra-abdominal sources of infection. Variants of SBP are:

(i) Classic SBP: ascitic fluid polymorphonuclear leucocyte (PMN) counts >250 /mm3 and positive culture.
(ii) Culture negative neutrocytic ascites (CNNA): ascitic fluid PMN counts >250 /mm3 and culture negative.
(iii) Bacterascites:- a culture positive ascitic fluid in the presence of PMN counts <250 /mm3. Its occurrence is related to low protein levels and impaired opsonic activity in ascitic fluid. Most episodes of spontaneous bacterial peritonitis are monomicrobial and produced by enteric bacteria.
(iv) Monomicrobial non neutrocytic bacterascites (MNB): Which includes positive ascitic fluid culture for single organism and ascitic fluid PNL less than 250 cells/ml and no evident intra-abdominal surgically treatable source of infection **[3].**

As this activity is also significantly related to the C3 concentration, it may be assumed that patients with low C3 concentration in ascitic fluid may be more susceptible to the development of SBP **[4].**

Several methods for the early diagnosis of SBP have been proposed, including the ratio of ascitic fluid pH to arterial blood pH, lactate concentration in the ascitic fluid, the gram stain and leucocyte count. The combination of two or more of these methods may lead to a more accurate diagnosis of this complication **[2].**

This study aimed to assess the association between serum and ascitic fluid levels of C3 in patient

with cirrhotic ascites complicated by spontaneous bacterial peritonitis.

Patients and Methods:

This study was performed on 50 patients admitted to Internal Medicine and Tropical Medicine Departments of Ain Shams University Hospitals. They were divided into three groups: **Group I**: 20 patients with cirrhotic ascites complicated by SBP (diagnosed by clinical picture, ascitic fluid neutrophilic count more than250 cells/mm³ and ascitic fluid culture with exclusion of patients with culture-negative neutrocytic ascites or culture-positive ascitic fluid in the presence of PMN counts<250 cells/mm³ i.e. Bacterascites). **Group II**: 20 patients with cirrhotic ascites not complicated by SBP. **Group III**: 10 patients with non-cirrhotic ascites (mesothelioma, abdominal lymphoma, nephrotic syndrome). Informed consent was obtained from all patients and also the approval of the ethical committee was done. Patients with raised serum C3 levels due to acute inflammatory reactions or patients with decreased serum C3 levels due to recurrent infections, systemic lupus erythematosis, glomerulonephritis and other conditions were excluded from the study.

All enrolled patients in the study were subjected to the following: Detailed history, thorough clinical examination, and laboratory investigations including: Complete Blood count (CBC), liver function tests, kidney function tests, prothrombin time, Serum electrolytes, Random blood glucose level, Complement levels of C3 analyzed by immunoperfusion in serum and ascitic samples using turbidimetery (Turbiquant C3, Behringwerke Diagnostics-Marburg, Germany). The aspirated ascitic fluid samples were examined for: polymorphonuclear leucocytic (PMN) count using hemocytometer and microscopic method, biochemical assay of glucose, total proteins, albumin and lactate dehydrogenase (LDH) and bacteriological culture using aerobic and anaerobic standard blood culture bottles, which were inoculated with 10 mL of ascitic fluid at the bedside and incubated for 48 hours at 37°C. Abdominal Ultrasonography was done to assess the presence of the ascites. Patients were classified according to Child's- Pugh score into Child's Class A, B and C **[5].** Informed consent was taken from all patients and also the approval of the ethical committee was done before being enrolled in the study.

Statistical Analysis:

Collected data were analyzed using SPSS (version 16) statistical software package under Windows XP operating system. Qualitative data were presented as frequency (number and %). Chi-square test was used for comparison of qualitative data. Quantitative data were presented as mean ± standard deviation (x ± SD). Paired t-test was used to compare parametric quantitative variables in the same group and Unpaired t-test was used to compare parametric quantitative variables in different groups. Spearman Correlation Coefficient (r) test was used to rank different variables against each other either positive or inverse. Receiver Operating Characteristic (ROC) curve was used to determine the best cut off values of best cutoff value of serum and ascetic fluid C3 for discriminating patients with SBP from patients without SBP with detection of sensitivity and specificity at this cutoff value. p value <0.05 and <0.01 was considered significant and highly significant, respectively.

Results:

Demographic and clinical data of the studied patients

This study was conducted on 50 patients (40 patients with liver cirrhosis and ascites and 10 patients with non cirrhotic ascites) included 33 male (66%) and 17 females (34%) with mean age 50.46±11.27, they were divided into three groups: group I (cirrhotic ascites complicated with SBP): 12 males (60%), 8 females (40%), with mean age 49.55±8.55, group II (cirrhotic ascites without SBP): 13 males (65%), 7 female (35%), with mean age 51.50±7.46, and group III (non cirrhotic ascites) (2 of them had mesothelioma, another 2 had nephrotic syndrome , another 2 had cancer colon and the last 4 had lymphoma.): 8 males (80%), 2 females (20%), with mean age 50.20±20.39. Comparison between groups revealed no statistically significant differences as regards age and sex.

Child's classification of studied patients:

Among the studied 40 patients with liver cirrhosis and ascites (group I and group II) 15/40 patients (37.5%) were Child's B and 25/40 patients (62.5%) were Child's C. In Group I 5 patients (25%) were Child's B and 15patients (75%) were Child's C, while in Group II 10 patients (50%) were Child's B and 10 patients (50%) were Child's C with no statistically significant difference between both groups (P=0.095). There was a highly statistically significant difference between Child's B and Child's C as regard serum C3 (P=0.004) however there was no statistically significant difference between Child's B and Child's C regarding ascitic C3 (P=0.934). (Table 1).

Serum C3 level in studied patients

Comparison between the level of serum C3 in the three groups, there was highly statistically significant difference between the three groups with (P= 0.001). By doing the post-hoc test to detect the least significant difference (LSD), it was found that

there was a highly statistically significant difference between the group I (with mean level 28.6±7.87) and group II (with mean level 80.15±55.27) with (P_1=0.00) and a statistically significant difference between the group I and group III (with mean level 63.6±52.33) with (P_2=0.038), while no statistically significant difference between the group II and III with (P_3 =0.317). (Table 2).

Ascitic C3 level in studied patients:

Regarding the level of ascitic fluid C3 there was statistically significant difference between the three groups with (P = 0.025). By doing the post-hoc test to detect LSD it was found that there was a statistically significant difference between the group I (with mean level 9.55±2.28) and group II (with mean level 17.4±13.71) with (P_1= 0.012) and between the group I and III (with mean level 17±7.83) with (P_2=0.048), but no statistically significant difference between the group II and III with (P_3= 0.914). (Table 2).

Ascitic Fluid Parameters in the studied patients

There was highly statistically significant difference between the three groups regarding the ascitic WBCs with (P= 0.000), also regarding the ascitic glucose level with (P= 0.00) and regarding the ascitic albumin level with (P= 0.001). However, regarding the ascitic LDH there was a statistically significant difference with (P= 0.020). (Table 2).

Correlation studies done on different parameters

There was a statistically significant positive correlation between serum C3 with s.alb while there was a highly statistically significant positive correlation between serum C3 and ascitic C3 but there was no statistically significant correlation between serum C3 with PT and TLC. (Table 3).

There was a statistically significant negative correlation between ascitic C3 with ascitic WBCs While there was no statistically significant correlation between ascitic C3 with ascitic glucose, ascitic albumin and ascitic LDH. (Table 4).

The diagnostic performance of serum C3 and ascitic C3 in patients with spontaneous bacterial peritonitis

Using ROC curve analysis showing that serum C3 of 45 g/L was the best cutoff value for discriminating patients with SBP from patients without SBP, with 85% sensitivity and 100% specificity, while showing that ascitic C3 of 11 g/L was the best cutoff value for discriminating patients with SBP from patients without SBP with 60% sensitivity and 90% specificity . (Figure 1).

Discussion:

Spontaneous bacterial peritonitis is a severe and frequent complication of cirrhosis with a high mortality rate. It is probably related to several impaired defense mechanisms, such as depressed reticuloendothelial system phagocytic activity, leucocyte dysfunction and reduced serum complement **[6].**

There is decreased antibodies and impaired bactericidal function of IgM in 80% of patients with cirrhosis. IgG, IgA and IgM levels in cirrhotic ascites were significantly lower than non-cirrhotic ascites. It has been postulated that massive ascites, which often occur in cirrhotic patients, dilutes these components **[3].**

C3 is the key marker for the classical and alternative pathway of complement activation. C4 is the marker for the classical pathway. Both are mainly produced in the liver **[7].**

Decreased serum complement concentrations, in particular C3 and C4 have been described in patients with liver cirrhosis **[8].** This is the result of two mechanisms: a failure to synthesize a certain number of components and regulatory proteins of complement and an increased consumption due to activation of the complement system. This acquired deficit in complement contributes to the increased risk of infection in patients with cirrhosis **[9].** Lower concentrations of C3 have also been reported in patients with advanced liver cirrhosis with SBP **[10].**

In the present study, there was a highly statistically significant difference in serum C3 level between Group I (cirrhotic ascities complicated by SBP) and Group II (cirrhotic ascites not complicated by SBP)where serum C3 was lower in Group I than in Group II, these results agreed with **Rabinovitz et al. [11]** who found that plasma C3 concentration were significantly reduced in patients with SBP compared with uninfected patients with (P <0.05).These results most probably due to increased complement activation rather than decreased hepatic synthesis.

There was a statistically significant difference in serum C3 level between group I (cirrhotic ascites complicated by spontaneous bacterial peritonitis) and group III (non-cirrhotic ascites due to other causes rather than liver cirrhosis) where serum C3 was lower in group I than group III with a (P=0.038). These results go with **Yildrim et. al. study [12]** in which, serum C3 levels were higher in Malignancy-induced ascites (ovarian cancers, hepatomas, colonic cancers, meshothelioma, gastric carcinomas, breast carcinomas, cholangiocarcinomas and pancreatic carcinoma) than in cirrhotic ascites with spontaneous bacterial peritonitis with a (P=0.006).

In the present study, there was no statistically significant difference between group II and group III (regarding serum C3 level) with (P= 0.317). This

result was consistent with **Rabinovitz et al. study [11]** in which the serum C3 concentration in patients without SBP was not significantly different from those in normal control subjects.

In **Rabinovitz** et al. **study [11]** this can be explained by the presence of many patients in non-SBP group with alcoholic cirrhosis superimposed with alcoholic hepatitis and that the increase in plasma C3 level could be secondary to increased synthesis as part of the acute phase response in patients with alcoholic hepatitis. In addition, this can be explained in this study by absence of exclusion criteria in choosing patients of Group III such as excluding patients with diseases associated with deficiency of C3 (i.e. SLE, Glomerulopathies, Vasculitis ,…etc).

In our study, it was found that there was a statistically significant difference in the level of ascitic C3 between the three groups with (P=0.025). There was a statistically significant difference between group I and group II where ascitic C3 was lower in group I than group II with (P=0.012).This results goes with **Rabinovitz** et al. **study [11].**

There was a statistically significant difference between group I and group III where ascitic C3 was lower in group I than group III with (P=0.048).This goes with **Yildrim et al study [12].**There was no statistically significant difference in ascitic C3 level between group II and group III with (P=0.914).

In this study, there was a highly statistically significant difference in serum C3 level between Child B and Child C where serum C3 is higher in Child B than Child C with (P=0.004). This can be explained by decreased biosynthesis of circulating complement proteins due to compromised liver function more in Child C .Also in Child C there is more impairment in hepatic reticuloendothelial system function with more susceptibility to SBP. No statistically significant difference found in ascitic C3 level between Child B and Child C with (P= 0.934), this agreed with **Baumann et al study [7]** who stated that Serum complement concentrations of C3 correlate negatively with the Child-Pugh score in patients with liver cirrhosis. C3 concentrations are lower in those Child-Pugh C cirrhosis patients.

In the present study, there was a positive correlation between serum C3 and ascitic C3 level with a (P=0.009).This goes with **Yildirium et al study [12]** which revealed a highly statistically significant correlation between serum C3 and ascitic C3 with (P= 0.007). On the other hand, there was a positive correlation between serum C3 with serum albumin. This can be explained by the more advanced liver disease, the more impaired hepatic biosynthesis with a decrease in serum albumin and C3 level.

Table (1): Comparison between Child's B and Child's C regarding the levels of serum and ascitic C3

	Child's B (N=15) Mean ± SD	**Child's C (N=25)** Mean ± SD	**F Value**	**P value**
Serum C3	73.26 ± 68.0	43.3 ± 22.74	9.48	0.004
Ascitic C3	1308 ± 6.46	13.28 ± 12.41	0.007	0.934

Table (2): Comparison the levels of different variables among the three groups

	Group I No. 20 mean± SD	Group II No. 20 mean± SD	Group III No.10 mean± SD	F value	P value	Post Hoc P value
Serum C3	28.6 ± 7.87	80.15±55.27	63.60±52.33	7.63	0.001	P_1 0.00 P_2 0.038 P_3 0.317
Ascitic C3	9.55 ± 2.28	17.4±13.71	17.0±7.83	3.98	0.025	P_1 0.012 P_2 0.048 P_3 0.914
Ascitic *WBCs*	318 ± 112	34.25±31.5	102.5±105.3	55.94	0.00	P_1 0.00 P_2 0.00 P_3 0.049
Ascitic *LDH*	172.5±172	148.6±126	521.4±740	4.24	0.020	P_1 0.83 P_2 0.014 P_3 0.009
Ascitic *GLU*	73.8 ± 27.2	120.8±35.3	106.6±12.64	13.63	0.00	P_1 0.00 P_2 0.005 P_3 0.211
Ascitic *ALB*	0.87 ± 0.2	0.36±0.2	1.1±1.0	8.47	0.001	P_1 0.003 P_2 0.266 P_3 0.79
SAAG	1.41 ± 0.52	2.07±0.45	2.01±0.89	6.96	0.002	$P_1$0.001 P_2 0.012 P_3 0.79

P_1 between group I and group II ; P_2 between group I and group III; P_3 between group II and group III

Table (3): Correlation between serum C3 level with S.ALB, PT, TLC, Ascitic C3:

No.50	S.ALB	PT	TLC	Ascitic C3
r-value	0.275	-0.261	-0.064	0.364
P-value	0.053	0.067	0.659	0.009

Table (4): Correlation between Ascitic C3 level and other Ascitic Fluid Parameters:

No.50	Ascitic *WBCs*	Ascitic *LDH*	Ascitic *GLU*	Ascitic *ALB*
r-value	-0.31	0.271	0.075	0.11
P-value	0.028	0.057	0.604	0.449

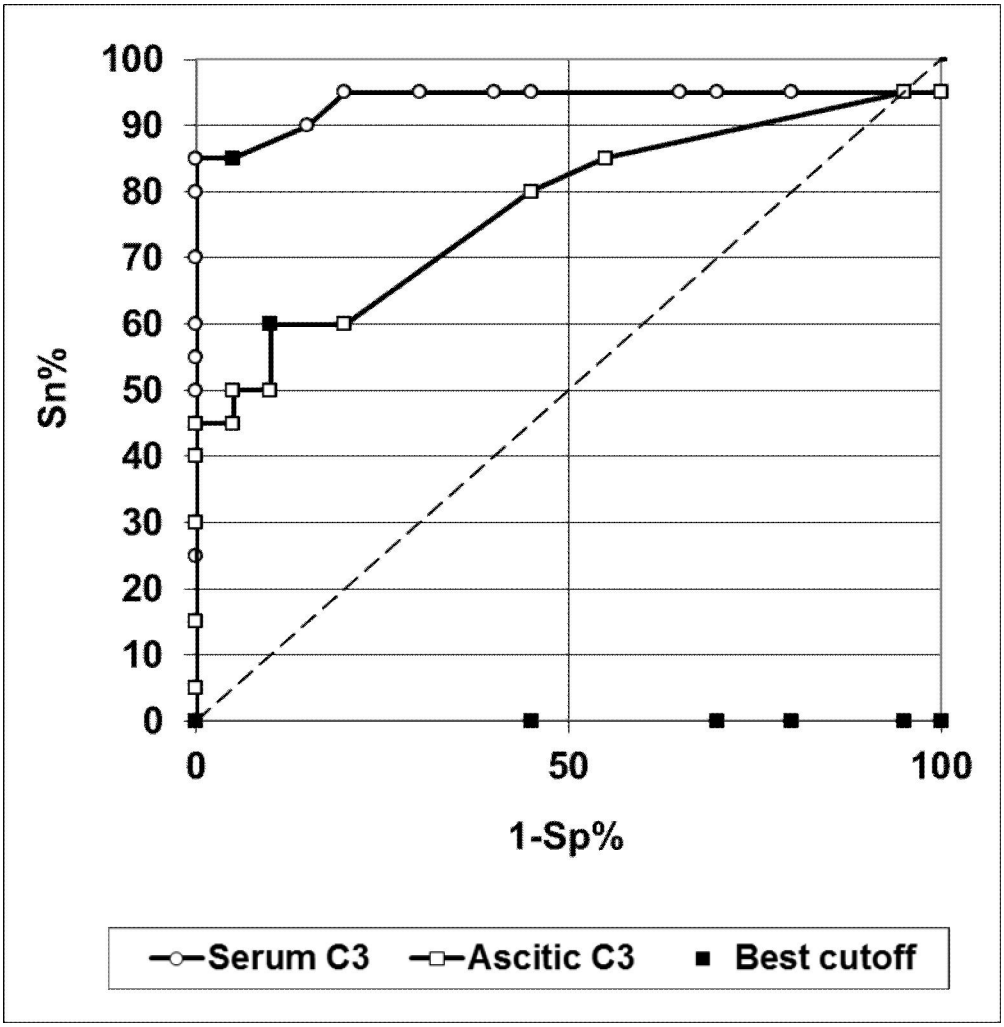

Figure (1): ROC curve analysis showing the diagnostic performance of Serum C3 and Ascitic C3 for discriminating patients with SBP and without SBP

Conclusion:

The deficiency of serum complement concentrations of C3 is influenced with more progress of the disease and presence of ascites complicated with SBP, so follow up of complement concentrations may help to recognize patients with increased risk for the development of SBP. Serum C3 of 45g/L and ascitic C3 of 11 g/L was the best cutoff value for discriminating patients with SBP from patients without SBP.

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