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RESEARCH ARTICLE

Serum Complement 3 Level and Culture Positivity As Predictors Of Spontaneous Bacterial Peritonitis Recurrence In Egyptian Cirrhotic Patients

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Abstract

Background: Spontaneous bacterial peritonitis (SBP) is one of the most common and life threatening complications of cirrhosis. The risk of SBP recurrence is around 70% per year. Altered host immune system and presence of infection are thought to be the basis for its recurrence.

Objective: To study serum complement 3 level and culture positivity as predictors of SBP recurrence in Egyptian cirrhotic patients.

Patients &Methods: This study was carried out at Faculty of Medicine, Zagazig University, Egypt. It is a prospective case-control study in which we recruited 160 cirrhotic ascitic patients presenting with SBP. Any episode of SBP after resolution of the first index case of SBP within 6months was considered as recurrence. They were divided after follow up for 6 months to detect recurrence into two groups; Group I: 79 cirrhotic ascitic patients without recurrent SBP (control group) and Group II : 71 cirrhotic ascitic patients with recurrent SBP (cases group). All patients were subjected to full history taking, general and local abdominal examinations and ultrasonographic examination. In addition, they were subjected to routine laboratory investigations, ascitic fluid (AF) analysis and culture and serum complement C3 level assay

Results: In the current study, there was 71 of 150 (47.3%) patients developed recurrent SBP. Recurrent SBP developed in patients with total bilirubin >1.2 mg/dL ($p < 0.001$), serum albumin < 3.5 g/dL ($p < 0.001$), SGOT > 35 IU/L ($p < 0.032$), ascitic protein <1 gm/dL ($p < 0.001$), PT >18 seconds ($p < 0.001$) and AF polymorphonuclear leucocyte count < 250 cell/ mm^3 ($p < 0.001$), serum C3 <90 mg/dL ($p < 0.001$) and positive culture of ascitic fluid ($p < 0.001$). Multivariate logistic regression analysis for the most predictor variables of recurrent SBP among the studied patients were decreased serum C3**,** positive culture, increased total bilirubin, decreased serum albumin, decreased AF protein and prolonged PT.

Conclusion: Recurrent SBP was noticed in 47.3% of Egyptian cirrhotic patients. Serum C3 <90 mg/dl and positive culture were significant factors associated with recurrent SBP in Egyptian cirrhotic patients.

Introduction

Spontaneous bacterial peritonitis (SBP) is one of the most common and life threatening complications of cirrhosis which is characterized by the infection of ascitic fluid (AF) in the absence of a concurrent intra-abdominal source of infection (e.g. intra-abdominal abscesses, intestinal perforation, cholecystitis or acute pancreatitis)**[1]**.

There was around 10% probability of developing SBP in patients with end stage liver disease and ascites over a period of one year **[2]**, this may related to several impaired defense mechanisms, such as depressed phagocytic activity, leukocyte dysfunction, reduced serum complement (C3), and low bactericidal activity of ascitic fluid **[3].**

Ascitic fluid C3 concentration and opsonic activites are important protective factors against SBP, but as the liver is the main site of synthesis of complement components and C3 is the main component of complement system so it affects the progress of SBP in liver cirrhosis **[4]**

Gram-positive bacteria were the predominant pathogens associated with SBP. Although *Enterococcus faecalis* and *Streptococci viridans* were the most common Gram-positive pathogens, *Staphylococcus aureus* (S. aureus) accounted for 25% of the Gram-positive bacteria **[5]**. Another study found that *Escherichia coli* (*E. coli*) was the most common isolate from AF culture **(6)**.

The risk of SBP recurrence is around 70% per year **(7)**. Recurrent episodes are associated with a high mortality rate and are most likely to occur in patients with an AF protein level of < 1 g/dL and prolonged prothrombin time **(8).** Other Internationally published data showed that increased serum bilirubin level **(9)**, derangement in serum creatinine, presence of hyponatremia **(10)**, and the use of proton pump inhibitors (PPI) **(11)** are the risk factors associated with SBP**.**

Up to our knowledge, this is the first study from Egypt which studied the factors that are mainly responsible for the recurrence of SBP.

The aim of this work was to study serum complement 3 Level and culture positivity as predictors of SBP recurrence in Egyptian cirrhotic patients.

Patients and Methods

Research design:

This study was carried out at the Internal Medicine and Medical Microbiology and Immuonology Departments, Faculty of Medicine, Zagazig University between January 2013 and February 2014.

Type of study: Prospective case - control study

Ethical consideration:

Approval for performing the study was obtained from the Internal Medicine and Medical Microbiology and Immuonology Departments, faculty of medicine, Zagazig University after taking Institutional Review Board (IRB) approval.

Patients:

One hundred and sixty cirrhotic ascitic patients with SBP were included in this study. They were admitted in the Internal Medicine ICU and inpatient department. They were given the appropriate treatment during their hospital stay in form of a combination of third generation cephalosporin and metronidazole intravenously as the standard treatment. Their average hospital stay was 7-10 days **(12)**.

After patients' discharge, we followed them weekly in outpatient clinic for 6 successive months by repeated clinical examination, abdominal ultrasonography (US), laboratory tests, AF analysis including culture and re-measurement of C3 level.

By the end of the study, the total number of patients completing 6 successive months follow-up visits in the outpatient clinic was 150 as a total number. Drop out was either due to death or failure to show up for the repeated examinations. We divided them according to absence and presence of SBP recurrence into two groups, Group I:79 cirrhotic ascitic patients without recurrent SBP (control group) and Group II : 71 cirrhotic ascitic patients with recurrent SBP.

Inclusion criteria:

Egyptian cirrhotic ascitic patients who were diagnosed by clinical, laboratory and abdominal US. The diagnosis of SBP was based on the presence of ascitic fluid (AF) polymorphonuclear leukocytes (PMNLs) count of ≥ 250 cells/mm^3 **(13)**.

Exclusion criteria:

Patients with secondary peritonitis were excluded. Moreover, 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 also excluded from the study. Patients who had hepatocellular carcinoma, portal vein thrombosis, hepatorenal syndrome and diabetes mellitus. This was due to their potential effect on protein synthesis and loss.

All enrolled patients in the study were subjected to full history taking including fever, abdominal pain, constipation, diarrhea, nausea, vomiting, history of gastrointestinal bleeding or hepatic encephalopathy. In addition, history of bilhareziasis, and drug intake of diuretic, antibiotics and PPI.

They were also subjected to full general and local abdominal examinations and routine laboratory investigations including; complete blood count (CBC), liver function tests, kidney function tests, urine analysis and culture, stool analysis, prothrombin time (PT), international normalized ratio (INR), serum lactate dehydrogenase (LDH) level, Hepatitis B surface antigen and Hepatitis C virus antibody.

Abdominal US with special comment on liver, spleen, kidney and amount of AF was performed.

Precautions before AF analysis:

Antibiotics should be stopped for 3 successive days (If patients with SBP were taken it before diagnosis) and give them immediately after taking ascitic fluid samples. Diuretics were routinely discontinued at the time of diagnosis of SBP and therapeutic paracentesis was not allowed during the study.

Laboratory work up of ascitic fluid:

Aspirated ascitic fluid samples were examined for PMNLs count using hemocytometer. Also, protein, glucose and LDH levels were estimated. Ascitic fluid to serum LDH ratio is greater than 0.4 in SBP patients. We calculated, in addition, AF to serum glucose ratio that may be < 1.0 in these patients.

Ascitic fluid cultures were performed by inoculating 10 ml of the AF samples into blood culture bottles **(Remaux, France)** at the bedside using a sterile technique and incubated for 48 hours at 37°C.The samples were moved to the microbiology laboratory immediately. Subcultures were done every 48 hours on blood, chocolate and MacConkey agar **(Oxoid, UK)** plates both aerobically and anaerobically using BD GasPack EZ Anaerobic container **(Becton and Pickinson, USA)**. Bacterial isolates were identified on the basis of colonial morphology, microscopic examination of Gram stained films of the different colonies and different conventional biochemical reactions for either Gram positive or negative bacteria **(14)**.

We considered resolution of SBP as reduction of the elevated AF PMNLs count to < 250/mm^3, negative culture in previously positive cases with resolution of the clinical manifestations (**15**).

Quantitative determination of serum complement 3 (C3):

It was measured by turbidimetry (**Vital diagnostics S.r.l. via Balzella 41/G/4, 47100 Forlì, ITALY Forti, Italy)** using anti-human C3 antibodies. Reference values for adults were between 90-180 mg/dL.

Statistical Methods

The collected data were presented, summarized, tabulated, and analyzed using the Statistical Package for Social Sciences (SPSS version 19.0)**.** Qualitative data were presented as frequency (number and %). Quantitative data were presented as mean ± standard deviation (± SD). Comparisons between groups were performed using Chi square for qualitative variables and the Student's t-test for quantitative variables. All the significant predictors of recurrent SBP of the univariate analysis were included in the multivariate regression model. The 95% confidence interval (CI) was calculated wherever found appropriate. P-values <0.05 and <0.01 were considered significant and highly significant; respectively.

Results

In the current study 160 cirrhotic ascitic patients with SBP were included. By the end of the study, the total number of patients completing 6 successive months follow-up visits in the outpatient clinic was 150. Among them, 67/150 were females (44.7%) and 83/150 were males (55.3%) and their ages were (35-82) years.

Patients were divided into two groups after follow up them for 6 months: Group I: 79/150 (52.7%) that included patients without recurrent SBP and Group II: 71/150 (47.3%) that included patients with recurrent SBP.

The clinical presentation of SBP of the 150 patients is highly variable, as regard symptomatic patients, there were about (66%) patients who suffered from fever, mental status alteration (60%), abdominal tenderness (43%) and others (14%). On the other hand, about (32%) of patients were asymptomatic.

There was no statistical significant difference between the two groups as regards age, sex, past history of bilharziasis, HCV and HBV infections, using of diuretics, antibiotics and PPI. Also, there was no significant difference between two groups concerning Child class B&C **(table 1)**.

Our results showed statistical significant difference between the two groups as regards total bilirubin >1.2 mg/dL ($p < 0.001$), serum albumin < 3.5 g/dL ($p < 0.001$), SGOT >35 IU/L ($p < 0.032$) and PT >18 seconds ($p < 0.001$) **(table 2)**.

In this study, there were statistical significant differences between the two groups as regards; AF protein<1 g/dL ($p < 0.001$), AF PMNLs count <250 cell/ mm^3 ($p < 0.001$) and serum C3 < 90 mg/dL ($p < 0.001$). Moreover, culture positivity reported statistical significant differences (70.4% versus 20.3%) ($p < 0.001$). Gram-negative enterobacteriaceae (E. coli and Klebsiella pneumoniae (K. pneumoniae)) accounted for 90% and 93.8% of cases of recurrent and non-recurrent SBP; respectively. The remaining 10% and 6.2% were caused by S. aureus. There were statistical significant differences in isolated E.coli and K.pneumoniae ($p< 0.001$and 0.004; respectively) but there was statistical non-significant differences in isolated S.aureus ($p=0.071$) **(table 3).**

Multivariate logistic regression analysis for the most predictor variables of recurrent SBP among the studied patients were decreased serum C3**,** positive culture, increased total bilirubin, decreased serum albumin, decreased ascitic fluid protein and prolonged PT **(table 4).**

Discussion

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 SBP in cirrhotic patients with ascites is as high as 18%, with 40–70% associated mortality **(16).** Moreover, the hospital mortality rate of around 30 to 50% (**17**). The risk of SBP recurrence is around 70% per year **(18**).On the other hand, long-term prophylaxis with antibiotics like quinolones has reduced the recurrence rate, but emergence of quinolone resistant organisms has lowered its use **(19)**.

There is insufficient regional and local data about factors responsible for the recurrence of SBP. Hence, we aimed in this study to detect factors predicting SBP recurrence among cirrhotic ascetic Egyptian patients focusing on serum complement 3 Level and culture positivity.

In our work, as regards demographic data (age, sex), there was statistically non-significant difference between patients without and with SBP recurrence (group I and group II). It was correlated with previous study (**20)** but another study showed significant difference between these groups as regards sex **(21)**.

Concerning drug history, our results disagreed with **Siple et al.** (**22**),who reported that use of PPI increasing the risk of SBP infection in cirrhotic ascitic patients. They postulated that the portal hypertensive environment in cirrhosis and decreasing the defense mechanism of gastric acidity as a result of using PPI could be increasing the risk of SBP.

In this work, clinical characteristics had statistically non-significant results and these were correlated with previous study **(20)** but in another study, **Jamil et al. (21**) found that HBV had statistically significant results. The possible mechanism may be altered immunity and deficiencies in complement system with depressed functions of neutrophils, macrophages and immunity in patients with cirrhosis (**21**).

In addition, our results determined statistically non-significant relationship as regards Child class (B&C) and this against results obtained by **Jamil et al. (21)**.

We found statistically significant difference concerning total bilirubin >1.2 mg/dL that was correlated with a Spanish study by **Titó et al. (20)** who found serum bilirubin > 4 mg per/dL associated with a high risk of recurrence of SBP on univariate analysis and also correlate with study of **Jamil et al. (21)** who found that serum bilirubin >1mg/dl associated with a high risk of recurrence of SBP on univariate and multivariant analysis.

Our results discovered that decreasing serum albumin increasing the chance for SBP recurrence,which agreed with **Huang et al.** (**23)**.

There was significant difference concerning prothrombin time more than 18 seconds that was correlated with **Titó et al. (20)** who showed decreased activity of prothrombin but did not correlate with study of **Jamil et al. (21)** .

In this study, we found statistically significant difference as regards ascetic fluid protein <1g/dL and that correlated with the study of **Titó et al. (20)** who reported that protein concentration in ascitic fluid <1g/dL was a predictor for SBP recurrence in the univariate and multivariate analysis.

Spontaneous bacterial peritonitis only appears in patients whose AF bacterial killing capacity is impaired. In this context, it is important to point out that the bactericidal and opsonic activity of ascites and the AF concentration of several defensive substances, such as immunoglobulins, complement and fibronectin, have been found to vary greatly affected in cirrhotics **(24, 25).** Besides that, Mustafa et al. **(26)** suggested that ascitic fluid protein level had statistically significant reducion in cirrhotic patients who develop SBP.

In particular, C3 had been described in patients with liver cirrhosis. 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 attributed to acquired deficit in complement that contributes to the increased risk of infection in patients with cirrhosis. Moreover, patients with advanced liver cirrhosis with SBP had lower concentrations of C3 **(27).**

In this study, we found a statistical significant low level of serum C3 < 90 mg/dL in recurrent SBP patients. This is in agreement with the Egyptian study of Kamal et al. **(12).** Moreover, **Cholongitas and co-workers** (**28**) considered low level of serum C3 as a predictor for recurrence of SBP, they had been proven that cirrhotic patients with a lower level of serum C3 and C4 are more prone to develop bacterial translocation**.**

In this research, as regards association of positive culture with recurrent SBP, there was a significant difference concerning this topic, as Gram-negative enterobacteriaceae (*E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*)) accounted for 90% and 93.8% of cases of recurrent and non-recurrent SBP; respectively. The remaining 10% and 6.2% were caused by *S. aureus*. This is in agreement with study of Kamal and co-workers **(12**) who designed it on patients with SBP. **Park et al. (29)**, also, found that Gram-negative organisms accounted for more than 60% of cases of SBP.

On contrary, a Chinese study documented that the overuse of antibiotics over the past 20 years, especially third-generation cephalosporins, may be causing the increasing frequency of Gram-positive bacteria in SBP cases in China (**30)**.

Conclusion: Recurrent SBP was noticed in 47.3% of Egyptian cirrhotic patients. Serum C3 <90 mg/dl and positive culture were statistically significant factors associated with its occurrence.

Recommendation:

We recommend regular follow up of patients with previous SBP for early diagnosis of recurrence using serum C3 as a routine laboratory investigation.

Table 1. Demographic and Clinical Characteristics of The Two Studied Groups.

	Group I n=79 n(%)	**Group II n=71 n(%)**	χ^2	**p-value**
Sex - Female - Male	35(44.3) 43(55.7)	32(45.1) 39(54.9)	0.02	0.888
Age in years Mean±SD (Range)	**Group I**	**Group II**	**T test**	**p-value**
	56.9±7.4 (35-72)	56.4±6.4 (35-82)	0.373	0.71
Bilharziasis	59(74.7)	46(64.8)	1.74	0.186
HCV	78(98.7)	69(97.2)	0.46	0.498
HBV	11(13.9)	7(9.9)	0.59	0.444
Use of diuretic	70(88.6)	65(91.5)	0.36	0.548
Use of antibiotic	11(13.9)	12(16.9)	0.26	0.613
Use of PPI	65(82.3)	40(56.3)	0.542	0.462
Child class **B** **C**	48 (60.8) 31(39.2)	50 (70.4) 21(29.6)	1.54	0.214

Table 2. Laboratory Investigations of The Two Studied Groups.

Variable		Group I n=79 n (%)	Group II n=71 n(%)	χ^2	p-value
WBC ($X10^3/mm^3$)	**> 4.0**	58 (73.4)	50 (70.4)	0.17	0.683
	< 4.0	21(26.9)	21(29.6)		
Hb(g/dL)	**> 12.0**	5(6.3)	5(7.0)	0.03	0.681
	< 12.0	74(93.7)	66(93.0)		
Platelet ($X10^3$/ mm^3)	**>150.0**	13(16.5)	9(12.7)	0.43	0.513
	<150.0	66(83.5)	62(87.3)		
Total bilirubin (mg/dL)	**Up to 1.2**	41(51.8)	7(9.9)	30.37	<0.001**
	> 1.2	38(48.1)	64(90.1)		
S. Albumin (g/dL)	**>3.5**	35(44.3)	2(2.8)	34.64	<0.001**
	< 3.5	44(55.7)	69(97.2)		
S.GPT(IU/mL)	**< 35.0**	38(48.1)	28(39.4)	1.41	0.285
	> 35.0	41(51.9)	43(60.6)		
S.GOT(IU/mL)	**< 35.0**	28(34.2)	14(19.7)	4.59	0.032*
	> 35.0	51(65.8)	57(80.3)		
PT(seconds)	**<18.0**	39(49.4)	9(12.7)	23.13	<0.001**
	> 18.0	40(50.6)	62(87.3)		
INR	**< 1.0**	4(5.1)	1(1.4)	1.55	0.213
	> 1.0	75(94.9)	70(98.6)		
S.Creatinine (mg/dL)	**Up to 1.4**	44(55.7)	30(42.3)	2.7	0.1
	> 1.4	35(44.3)	41(57.7)		
Urinary tract infection		67(84.8)	61(85.9)	0.04	0.848

Table 3. Comparison Between Both Groups as Regards AF Examination, Serum C3 Level and Culture Positivity.

Variable		GroupI n=79 n(%)	GroupII n=71 n(%)	χ^2	P
AF protein (g/dL)	**<1**	18(22.8)	66(93.0)	74.73	<0.001**
	>1	61(77.2)	5(7.0)		
AF/Serum glucose ratio	**<1**	68(86.1)	62(87.3)	0.05	0.822
	>1	11(13.9)	9(12.7)		
AF PMNLs count (cell/mm^3)	**<250**	79(100.0)	0(0.0)	150	<0.001**
	>250	0(0.0)	71(100.0)		
AF/Serum LDH ratio	**> 0.4**	37(46.8)	30(42.3)	0.32	0.573
	< 0 .4	42(53.2)	41(57.7)		
Serum C3 level (mg/dL)	**<90**	31(39.2)	70(98.6)	59.88	<0.001**
	>90	48(60.8)	1(1.4)		
AF Culture	**Negative**	63 (79.7)	21(29.6)	38.2	<0.001**
	Positive	16(20.3)	50(70.4)		
	E. coli	10 (62.5)	29(58.0)	15.44	<0.001**
	K.pneumoniae	5 (31.3)	16(32.0)	8.16	0.004*
	S. aureus	1 (6.2)	5(10.0)	3.25	0.071

Table 4. Multivariate Logistic Regression Analysis for The Most Predictor Variables of Recurrent SBP Among The Studied Patients.

Predictor variable	95% C.I. for EXP(B)	P

	Lower	Upper	
Serum C3<90(mg/dL)	1.44	311.88	0.026*
Positive culture	1.082	35.837	0.041*
Total bilirubin >1.2(g/dL)	28031	137269	<0.001**
S. albumin <3.5 (g/dL)	3.122	242.385	0.003*
Ascitic protein <1(g/dL)	5.555	239.757	<0.001**
PT >18(seconds)	1.518	65.247	0.017*

References

1. **Rimola A, García-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, et al.** Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. J Hepatol 2000; 32:142-53.
2. **Ribeiro TC, Chebli JM, Kondo M, Gaburri PD, Chebli LA, Feldner AC.** Spontaneous bacterial peritonitis: how to deal with this life-threatening cirrhosis complication? Ther Clin Risk Manag 2008; 4: 919-25.
3. **Yildirim B, Sari R, Sezgin N.** Complement and immunoglobulin levels in serum and ascitic fluid of patients with spontaneous bacterial peritonitis, malignant ascites, and tuberculous peritonitis. South Med J 2002;29:1158–62.
4. **Wyke RJ, Rajkovic IA, Eddleston AL, Williams R.** Defective opsonisation and complement deficiency in serum from patient with hepatic failure.GUT 1980; 21(8):643-9.
5. **Singh N, Wagener MM, Gayowski T.** Changing epidemiology and predictors of mortality in patients with spontaneous bacterial peritonitis at a liver transplant unit. Clin Microbiol Infect 2003; 9: 531-7.
6. **Kamani L, Mumtaz K, Ahmed US, Ali AW, Jafri W.** Outcomes in culture positive and culture negative ascitic fluid infection in patients with viral cirrhosis: cohort study. BMC Gastroenterol.2008; 18;8:59.
7. **Ginès P, Cárdenas A, Arroyo V, Rodés J.** Management of cirrhosis and ascites. N Engl J Med 2004; 350:1646-54.
8. **Soriano G.** Spontanous bacterial peritonitis. Semin Liver Dis 1997; 17(3): 203-17.
9. **Guarner C, Runyon BA.** Spontaneous bacterial peritonitis: pathogenesis, diagnosis, and management. Gastroenterologist 1995; 3:311-28.
10. **Porcel A, Diaz F, Rendon P, Macias M, Martin-Herrera L, Giron- Gonzalez JA.** Dilutional hyponatremia in patients with cirrhosis and ascites. Arch Intern Med 2002; 162:323-8.
11. **Garcia Rodriguez LA, Ruigomez A, Panes J.** Use of acid-suppressing drugs and the risk of bacterial gastroenteritis. Clin Gastroenterol Hepatol 2007; 5: 1418-23.
12. **Kamal AM, Osman E N,and Shahin R Y.** Role of ascitic fluid C3 in spontaneous bacterial peritonitis**.** The Egyptian Journal of Medical Human Genetics 2012; 13; 81-5**.**
13. **European Association for the Study of the Liver.** EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. J Hepatol 2010;53:397–417.
14. **Forbes BA, Sahm DF and Weissfeld AS (2007):** Overview of bacterial identification, methods and strategies. In: Baily and Scott's Diagnostic Microbiology. Belly A and Forbes B (eds). 12th ed. Mosby, New York; 221-45.
15. **Badawy AA, Zaher TI, Sharaf SM, Emara MH, Shaheen NE, and Aly TF.** Effect of alternative antibiotic in treatment of cefotaxime resistant spontaneous bacterial peritonitis. World J Gastroenterol 2013; 19(8):1271-7.
16. **Golam M, Shahinul A, Mobin K, Korshed A, Salimur R, Nooruddin A, et al.** Study on ascitic fluid complement 3 level in cirrhotic patients with spontaneous bacterial peritonitis and without spontaneous bacterial peritonitis. Int J Gastroenterol 2007; 6: 1-3.
17. **Ginés P, Rimola A, Planas R, Vargas V, Marco F, Almela M, et al.** Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double-blind, placebo-controlled trial. Hepatology 1990; 12:716-24.

18. **Ginès P, Cárdenas A, Arroyo V, Rodés J** Management of cirrhosis and ascites. N Engl J Med 2004; 350:1646-54.
19. **Fernández J, Navasa M, Gómez J, Colmenero J, Vila J, Arroyo V, et al.** Bacterial infections in cirrhosis: epidemiological changeswith invasive procedures and norfloxacin prophylaxis. Hepatology 2002; 35:140-8.
20. **Tito L, Rimola A, Gines P, Lliach J, Arroyo V, Rodes J** Recurrence of spontaneous bacterial peritonitis in cirrhosis: frequency emand predictive factors. Hepatology 1988; 8: 27-31.
21. **Jamil S, Ahmed S, Memon A, Masood S, et al.** Factors predicting the recurrence of Spontaneous Bacterial Peritonitis in patients with Cirrhosis. J Coll Physicians Surg Pak 2011; 21(7): 407-410.
22. **Siple JF, Morey JM, Gutman TE, Weinberg KL, Collins PD.** Proton pump inhibitor use and association with spontaneous bacterial peritonitis in patients with cirrhosis and ascites. Ann Pharmacother. 2012;46(10):1413-8.
23. **Huang CH, Lin CY, Sheen IS, Chen WT, Lin TN, Ho YP, Chiu CT.** Recurrence of spontaneous bacterial peritonitis in cirrhotic patients non-prophylactically treated with norfloxacin: serum albumin as an easy but reliable predictive factor. Liver Int. 2011; 31(2):184-91.
24. **Runyon BA**. Patients with deficient ascetic fluid opsonic activity are predisposed to spontaneous bacterial peritonitis. Hepatology 1988;8:632-5.
25. **Navasa M , Rimola A , Rodes J .**Bacterial infections in liver disease. Sem Liv Dis 1997; 14:323-233.
26. **Mustafa MG , Al Mamun MA, Alam AK.** Study on ascitic fluid protein level in cirrhotic patients with spontaneous bacterial peritonitis. Bangladesh Med Res Counc Bull.2009; 35(2):41-3.
27. **Such J, Runyon A.** Spontaneous bacterial peritonitis. Clin Infect Dis 1998; 27: 669-6.
28. **Cholongitas E, Papatheodoridis GV, Lahanas A, Xanthaki A, Kontou-Kastellanou C, Archimandritis AJ.** Increasing frequency of Gram-positive bacteria in spontaneous bacterial peritonitis. Liver Int 2005; 25:57-61**.**
29. **Park MK, Lee JH, Byun YH.** Change in the profiles of causative agents and antibiotic resistance rate for spontaneous bacterial peritonitis: an analysis of cultured microorganisms in recent 12 years. Korean J Hepatol 2007;13(3):370-7.
30. **Gou Y, Zhang F, Liang Z, Bia X, Pan L,Wang W,Liu B,Wang J, and Wang.** Pathogens change in spontaneous bacterial peritonitis patients with cirrhosis. Afr. J. Microbiol.2011; 5(1) 1-7.