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Effect of Culture Technique on the Work Up in cirrhotic Patients with Spontaneous Bacterial Peritonitis

Usama Ahmed Arafa[1], Mahmoud Kamal El Samman[1], Laila Mohammed Yousef [2,] Abdalla Rashad Mohammed[1], Ali Mahmoud Ahmed Kassem[1].

1- Internal Medicine Department, Sohag Faculty of Medicine ,Sohag University.
2- Clinical Pathology Department, Sohag Faculty of Medicine ,Sohag university.

Background: Spontaneous bacterial peritonitis (SBP) is a serious complication in patients with advance liver cirrhosis and is associated with significant mortality. Multidrug resistance is an evolving problem in management of SBP. Therefore, early diagnosis and proper selection of antimicrobial therapy are warranted.

Objective: Assessment of the accuracy of conventional culture compared to blood culture in diagnosis of SBP and evaluation the role of blood culture in selection of antimicrobial therapy for treatment of SBP.

Methods: One hundred unselected consecutive cirrhotic patients with moderate or severe ascitis who were admitted to Internal Medicine Department during the period from October 2016 to April 2017 were included. Diagnostic aspiration of the ascetic fluid was made for each patient. The aspirated samples underwent chemical and cytological analysis as well as inoculation on conventional culture and on blood culture. Positive growths were tested for antibiotic sensitivity.

Results: 47 patients (47%) among the 100 cirrhotic patients had spontaneous bacterial peritonitis. Positive growths were detected in 11 patients (23.4%) and in 32(68.1%) patients by using conventional culture and blood culture respectively. By using blood culture as gold stander, the sensitivity, specificity, positive predictive value and negative predictive value of conventional culture were 34.38%, 100%, 100% and 41.67% respectively. All isolated growths were sensitive to meropenem. Resistance to cefotaxime was detected in 20 cases (62.5%). Other tested drugs showed variable degrees of sensitivity.

Conclusion: Conventional culture is of low sensitivity in diagnosis of SBP among cirrhotic patients and blood culture should be considered the gold standard for diagnosis of SBP. Multidrug resistance in SBP is common and antibiotic selection should be based on culture and sensitivity tests.

Key words: Spontaneous Bacterial Peritonitis, Blood Culture, Multidrug resistance.

Introduction:

Spontaneous bacterial peritonitis (SBP) is a serious infection caused by bacteria in cirrhotic patients and is associated with significant high mortality (1). There are various clinical local and/or systemic signs for SBP, ranging from local abdominal features of peritoneal inflammation with or without manifestations of systemic inflammation to impaired renal function without explanation (2). Early diagnosis and proper management of SBP have an important role in reduction of the morbidity and mortality that are associated with this serious infection in patients with advanced liver disease.

Ascitic fluid culture has an important role in the diagnosis and management of SBP. Conventional bacterial culture methods effectively detect bacteria in less than 50% of ascites samples with an elevated PMN count(>250/mm). The culture-positive rate of SBP is approximately 80% of cases assessed using the culture bottle method (3). Therefore, inoculation of the ascitic fluid into blood culture bottles at the patient's bedside is expected to increase the sensitivity of the bacterial culture.

Cefotaxime, a third-generation cephalosporin, was considered to be the drug of choice in treatment of SBP with a dose of 4 g/day for at least 5

days, this dose achieves high ascitic fluid concentrations and covers most of the causative organisms (4). Intestinal decontamination can also be achieved by other antibiotics. However, the extensive long-term use of these drugs lead to increased incidence of drug resistance and emergence of SBP caused by Gram-positive organisms (5). The emergence of resistant to bacteria gives the challenges in SBP treatment. Therefore, appropriate selection of antibiotic therapy for SBP is mandatory.

The aim of the study was to assess the use of conventional and blood cultures in management of SBP in patients with liver cirrhosis and their role in selection of the appropriate antibiotic therapy for treatment of SBP.

Materials and Methods:

The study was a prospective study that was conducted on cirrhotic patients who were admitted in Internal Medicine Department, Faculty of Medicine, Sohag University, during period from October 2016 to April 2017 and fulfilled the inclusion criteria. The study included cirrhotic patients with moderate or tense ascites, aged > 18 years, who accepted participation in this study. The exclusion criteria included presence definite alternative diagnosis for ascites, septic peritonitis, patients with burst abdomen or recent abdominal trauma and those who received antibiotic within 3 days before performing diagnostic paracentesis.

Diagnosis of liver cirrhosis was based upon clinical evaluation, liver function tests, abdominal ultrasonography, and hepatitis markers or Polymerase Chain Reaction (PCR). Diagnosis of SBP was conducted according to international guidelines if the polymorphonuclear leukocyte (PMN) cell count in the ascetic fluid exceeded 250/ml in the absence of source of intra-abdominal infection (6).

Clinical evaluation for all patients was done, including full history and clinical examination. Laboratory investigation included the following: Complete blood count (CBC) test was performed on Cell Dyn 3700 automated cell counter ,Abbott diagnostic. kidney function tests and liver function tests were done by Roche/Hitachi Coabas C311 system. Prothrombin time and concentration, inter-national normalization ratio (DadeBehring, Marburg, Germany kit). Blood culture of aspired fluid on Bact/ALERT SA Biomerieux. Under aseptic condition, diagnostic aspiration of the ascetic fluid was performed at the time of admission. The collected ascetic fluids were analysis (cells, protein and glucose) and inoculated on both conventional and blood culture. The isolated bacteria was tested for antimicrobial sensitivity. Each patient assigned a written informed consent and the study protocol was approved from our university ethics committee.

Statistical analysis:

Data was analyzed using STATA intercooled version 12.1. Data expressed as mean, standard deviation (SD), number and percentage. Mean and standard deviation were used as descriptive value for quantitative data. Chi square test was used for qualitative data. P value was considered significant if it was less than 0.05. When the data was not normally distributed Mann-Whitney test was used.

Results:

Clinical characteristic of the patients: The study involved 100 cirrhotic patient including54 (54%) male and 46(46%) female with mean age 62.02±10.32and range (30-86) year. The etiology of liver cirrhosis was HBV in 7(7%)patient, HCV in 42 (42%) patient and the remaining 51 (51%) patients were negative for HCV and HBV. Child Pugh score was Child B in 57(57%) patient and Child C in 43(43%) patient. Model For End-Stage Liver Disease (MELD score) mean was 12.71±2.9 and range (10-18). 84(84%)patient were presented by fever, and84 (84%)were presented by abdominal pain. Among included patients, 47 (47%) patients had SBP as shown in Table 1.

Conventional and blood cultures:

Among patients with SBP, blood culture of ascetic fluid showed positive growth in 32 case (68.08%) case and culture-negative neutrocytic ascites was detected in 15(31.9%) case. While, conventional culture revealed positive growth in 11(23.4%) case and it was negative in 36(76.6%) case. Compared to blood culture, conventional culture showed low sensitivity for diagnosis of SBP as shown in Table 2. Antimicrobial culture sensitivity was assessed using 15 drugs that suspected to be effective in treatment of SBP. Among these drugs, only meropenem showed sensitivity in all isolated growth. Cefotaxime, commonly used in treatment of SBP, showed intermediate antimicrobial sensitivity in 12 (37.5%) case and resistance in 20 (62.5%) case. Other examined drugs showed variable degrees of efficacy as shown in Table 3.

Table 1- Clinical data of all patients and patients with SBP

Variables	All patients (N=100)	Patients with SBP (N=47)
Age Mean ± SD / Median (range)	62.02±10.32 / 61 (30-86)	60.96±9.94 / 60 (40-85)
Gender Females / Males	46 (46 %) / 54 (54%)	20 (42.55 %) / 27 (57.45%)
Cause of liver cirrhosis HBV / HCV Others	7(7%) / 42 (42%) 51 (51 %)	3(6.38%) / 20 (42.55%) 42 (51.06 %)
Child Pugh score Child B / Child C	57(57%) / 43 (43%)	27 (57.45%) / 20 (42.55%)
MELD score Mean ± SD / Median (range)	21.7±2.9 / 14 (10-18)	12.6±3.06 / 10 (10-18)
Fever No / Yes	16(16%) / 84(84%)	9 (19.15%) / 38(80.85%)
Abdominal pain No / Yes	16 (16%) / 84(84%)	6 (12.77%) / 41 (87.23%)
Hepatic encephalopathy No / Yes	52 (52%) / 48 (48%)	25 (53.19%) / 22(48.81%)
History of SBP No / Yes	85 (85%) / 15 (15%)	34 (72.34%) / 13 (27.66%)
Presence of SBP	47 (47%)	47 (100%)
Conventional culture	11 (11%)	11 (23.4%)
Blood culture	32 (32%)	32 (68.08%)

SBP, spontaneous bacterial peritonitis: HBV, hepatitis B virus; HCV, hepatitis C virus; MELD, Model for End-Stage Liver Disease;

Table 2-Accuracy of conventional culture compared to blood culture:

Blood culture				
Conventional culture		Positive	Negative	Total
	Positive	11	0	11
	Negative	21	15	36
	Total	32	15	47
Sensitivity		34.38%		
Specificity		100%		
Positive predictive value		100%		
Negative predictive value		41.67%		
Accuracy		67.19%		

Table 3 - Antibiotics sensitivity of positive bacterial growth in blood culture.

Drugs	Antimicrobial Sensitivity		
	Sensitive	**Intermediate**	**Resistance**
Amoxicillin/ Clavulonic A	10 (31.25%)	14 (43.75%)	8 (25%)
Amikacin	18 (56.25%)	13 (40.63%)	1 (3.13%)
Chloramphen-icol	11 (34.38%)	18 (56.25%)	3 (9.38%)
Cefotaxime	0	12 (37.50%)	20 (62.50%)
Nitrofurantoin	14 (43.75%)	18 (56.25%)	0
Meropenem	32 (100%)	0	0
Neomycin	13 (40.63%)	17 (53.13%)	2 (6.25%)
Ampicillin / Sulbactam	17 (53.13%)	7 (21.88%)	8 (25%)
Tetracycline	9 (28.13%)	18 (56.25%)	5 (15.63%)
Levofloxacin	12 (37.50%)	12 (37.50%)	8 (25%)
Gentamycin	6 (18.75%)	21 (65.63%)	5 (15.63%)
Cefoprazole /Sulbactam	8 (25%)	15 (46.88%)	9 (28.13%)
Erythromycin	4 (12.50%)	18 (56.25%)	10 (31.25%)
Trimethoprim	5 (15.63%)	9 (28.13%)	18 (56.25%)
Sulfamethox-azole	3 (9.38%)	10 (31.25%)	19 (59.38%)
Rifampicin	2 (6.25%)	12 (37.50%)	18 (56.25%)

Discussion:

SBP is a serious complication that occurs in patients with advanced liver disease. Small intestinal bacterial overgrowth showed high prevalence among cirrhotic patients (7) and it has been associated with the development of SBP because of the bacterial translocation of from the intestinal lumen to the ascetic fluid and the systemic circulation as well (8). In our study, SBP was detected in 47 patients including 27 patients with Child B and 20 with Child C. High incidence of SBP among our patients could be due to special attention to diagnosis SBP in our department and performing diagnostic aspiration of ascetic fluid to all cirrhotic patients with ascites upon admission. Component of ascetic fluid bacteria is correlated with the stage of

hepatic decompensation and the Chid-Pugh score. The relationship is proving the link between gut microbiota and progression of cirrhosis (9).

Conventional culture of aspirated ascetic fluid in our study showed low sensitivity and low diagnostic accuracy compared to the use of blood culture bottles in diagnosis of SBP. In our study, we adapted bedside inoculation of ascitic fluid into blood culture bottles to increase the culture-positivity of the aspirated ascitic fluid. The results of ascetic fluid culture are affected by several factors including the use of blood culture bottles and the volume of the ascetic fluid sample.SBP is a low-colony-count monomicrobial infection which is similar to bacteremia (10,11,12). Several studies revealed that immediate inoculation of the aspirated ascitic fluid into blood culture bottles is superior to delayed inoculation in cirrhotic patients with ascitic fluid PMN count ≥250cells/mm^3 Culture positivity with immediate inoculation was reported to increase up to 80-100 % compared to 50-77% with delayed inoculation (13,11,12). Therefore, dramatic decreases in the sensitivity of the culture that can occur by sending of the aspirated fluid to the laboratory for culture should be avoided by following beside inoculation rather than delayed inoculation technique.

In our study, we inoculated 5 ml of aspirated ascetic fluid into 30 ml blood culture bottles. Runyon BA et al reported that inoculation of 10 or 20 mL of ascetic fluid into 100 mL blood culture bottles led to dramatic increase in culture-positivity rate than a 1 mL inoculum (93 versus 53 %) (10).

Culture-negative neutrocytic ascites was detected in about 30% of our cirrhotic patients with SBP and ascitic fluid PMN count ≥250cells /mm^3. Lack of delectation bacterial growth in presence of increased PMN count in ascetic fluid could be attributed to several factors. Inappropriately parenteral antibiotic administration before perfo-rming paracentesis is not infrequent and result in reduction in the culture positivity(14).Inadequate culture technique and inadequate volume are also included in reduction of culture positivity. Moreover, one of the other causes of culture-negative neutrocytic ascites could be overlooked in our study. Therefore, proper culture technique and avoiding antibiotic administration before paracentesis and proper exclusion of other causes of increased PMN in ascetic fluid are factors that should be considered to increase bacterial growth and culture positivity.

Third-generation, broad-spectrum cephalosporins were considered to be the agents of choice for treatment of SBP due to their superiority in randomized controlled trials and rare side effect profile and low risk of nephrotoxicity compared to other antibiotics (15,16,17). Cefotaxime was considered to be the drug of choice in treatment of SBP. It was reported to cover most SBP pathogens with excellent ascitic fluid penetration and it was associated with high sterilization rate (18). However, long term use of this drugs results in development of drug resistance organisms that are in need for new antibiotic or antibiotic combination to be resolved. Alternative intravenous antibiotic regimens for SBP other than third-generation cephalosporins are available but their use is associated with increased risk for adverse events and there is less evidence that support their role in primary treatment of SBP.

Our study showed poor sensitivity to several antibiotic that are frequently used in our community. Inappropriate

use of these drugs results in development of multi-drug-resistant bacteria which are expected to increase the morbidity and mortality in cirrhotic patients with SBP. Use of broad-spectrum antibiotic should be narrowed and should be based upon culture results to minimize the risk of development of antibiotic resistance.

We faced some limitations in our study. *Firstly*, the relative limited number of included patients with SBP. *Secondly*, lack of assessment antimicrobial sensitivity of the recent discovered broad spectrum antibiotics like glykopeptides and carbapenems which were considered to be an effective alternative therapy in treatment of SBP (19, 20).*Lastly*, lack of follow-up of recurrence of SBP among the treated patients. However, our study is the first study in our locality which highlight the importance of blood culture in treatment of SBP and proper selection of the effective antimicrobial therapy.

We concluded that blood culture of ascetic fluid in cirrhotic patient has an essential role in diagnosis of SBP in while conventional culture showed low sensitivity in the diagnosis. Multidrug resistance in SPB is common especially for cefotaxime and antibiotic selection should be based on antimicrobial culture sensitivity.

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