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BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN OF ASCITIC FLUID OF SUSPECTED SPONTANEOUS BACTERIAL PERITONITIS IN DECOMPENSATED CIRRHOSIS PATIENTS AT A TERTIARY CARE HOSPITAL, SOUTH INDIA

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ABSTRACT

BACKGROUND: Spontaneous bacterial peritonitis (SBP) has high mortality in cirrhotic patients. The occurrence of SBP is independent of the etiology of liver disease with prevalence varying from 8-36%.Hospital mortality of an episode of SBP can be reduced to 20% from 50% with appropriate antibiotics[1].

AIMS AND OBJECTIVES: To determine the Microbiological spectrum of SBP and to determine the antibiotic sensitivity pattern in culture positive, spontaneous bacterial peritonitis (SBP) patients.

METHODOLOGY: The present study is a prospective study conducted in tertiary care government general hospital, Kurnool, Andhra Pradesh for two years from March 2017 to March 2019. Two hundred patients with ascites, due to chronic liver disease admitted in the department of gastroenterology after considering the inclusion and exclusion criteria. Blood investigations and ascitic analysis, culture and other investigations done according to the predetermined protocol.

Results: Out of 200 patients included in study 58 (29%), patients had SBP. Most common organism identified is E.coli (54%) followed by Klebsiella pneumonia (16.6%), Pseudomonas auriginosa (12.5%) and Proteus mirabilis, Staphylococcus aureus (8.3%). E.Coli strains were resistant to cefotaxime, amikacin, ciprofloxacin, piperacillin-tazobactam, cefoperazone sulbactam in 30.7%,30.7%,38.5%,15.3%,23% of cases respectively. 25% of Klebsiella,33% of Pseudomonas auriginosa and 50% Staphylococcus aureus isolates were was resistant to cefotaxime, amikacin, ciprofloxacin, piperacillin-tazobactam, cefoperazone sulbactam antibiotics. All the isolated organisms were found to be sensitive to carbapenem antibiotic.

CONCLUSION: SBP prevalence found to be more common in patients with advanced liver disease with low ascitic and serum albumin levels. Most common organism identified is E.coli followed by Klebsiella, Pseudomonas, Proteus, Staphylococcus. Cefotaxime or piperacillin tazobactam to be choosen as empirical antibiotic.A high index of suspicion for SBP in cirrhotic patients and ascitic fluid analysis including culture will aid in the treatment and reduce the mortality related to SBP.

KEYWORDS :

OBJECTIVE OF STUDY:

1) To determine the Microbiological spectrum, antibiotic sensitivity pattern in culture positive, spontaneous bacterial peritonitis (SBP) patients with Cirrhosis and Ascites.

MATERIALS & METHODS

Study place:

This study was conducted in Tertiary Care Government General Hospital, Kurnool, Andhra Pradesh. All patients with ascites, due to chronic liver disease admitted in the Department of Gastroenterology ward were included in the study.

Period of study: 1.5 years
Number of cases: 200
Design of the study: A prospective observational study.

INCLUSION CRITERIA:

- Patients admitted in the Department of Gastroenterology at Govt. General Hospital, Kurnool and diagnosed to have decompensated chronic liver disease with ascites were included in the study.

EXCLUSION CRITERIA:

- Ascites due to renal, cardiac, tubercular, malignant pathology
- Secondary peritonitis
- Who are not willing to give consent
- Who received antibiotics within five days before admission
- Written and informed consent was taken from all the patients included in the study or close relatives where relevant.

METHOD OF COLLECTION OF DATA:

Basic demographics, symptoms, relevant clinical signs of patients were recorded. On admission day, before starting an empirical antibiotic, 30 ml of Ascitic fluid was collected at bedside using standard and universal precautions to ensure that a sterile sample is collected. Ten ml of ascitic fluid was added to EDTA tube for total, and differential counting and 5 ml was added to plain container for sugar, protein and albumin .15ml of ascitic fluid was injected into the blood culture bottle and culture positive Isolates were tested for antimicrobial susceptibility according to the type of bacteria isolated by using the disc diffusion method of modified Kirby Bauer.

Blood samples for CBP, Liver function tests, urea, creatinine, PT, INR, electrolytes were taken simultaneously.USG abdomen was done in all patients and UGI endoscopy done in patients who are stable to undergo the procedure. According to ascitic fluid cell count and culture results, patients were stratified into one of the three variants of SBP.

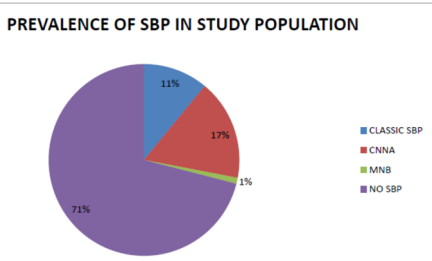
RESULTS

The total study group included 200 patients with cirrhosis and ascites. Of these 200 patients, 140 (70%) patients were males, and 60 (30%) were females. A male preponderance was seen in this study group as the main etiology of the cirrhosis is ethanol related.

PREVALENCE AND CLASSIFICATION OF SBP:

Out of the 200 cases of study population 58 (29%) were having SBP. Among these 22 cases were (37.9%) classic SBP with ascitic fluid PMN count >250/mm3 and culture being positive.34 cases (58.6%) were having CNNA (culture negative neutrocytic ascites with PMN count >250/mm3).

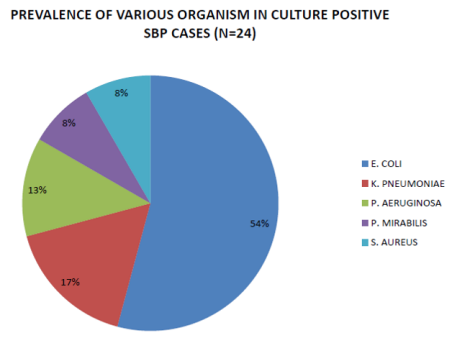
2 cases were having MNB (monomicrobial non neutrocytic ascites with culture being positive and PMN <250/mm3.



CULTURE AND SENSITIVITY OF SBP CASES:

All patients included in the study were investigated for SBP at the time of admission and ascitic fluid culture also sent at the time of admission. Of all cases of SBP (58), 24 cases were culture positive (41.3%). Antibiotic sensitivity pattern determined in culture positive cases. Most common organism identified is E.coli in 13 cases (54%) followed by Klebsiella pneumonia in 4 cases (16.6%), Pseudomonas aureginosa in 3 cases (12.5%) and Proteus mirabilis, Staphylococcus aureus in 2 cases each (8.3%). Two cases of MNB (monomicrobial nonneutrocytic bacterascites) were E.coli.

Fig-1: Prevalence Of Various Organisms In Culture Positive Sbp Cases(n=24)



Antibiotic Sensitivity In Culture Positive Cases :

- Of all cases (13) of E.Coli 4 cases (30.7%) were resistant to cefotaxime and amikacin, 5 cases (38.5%) resistant to ciprofloxacin, 2 cases(15.3%) resistant to piperacillin-tazobactam, 3 cases (23%) were resistant to cefoperazone sulbactam.
- Of all cases (4) of Klebsiella, one case (25%) was resistant to cefotaxime, amikacin, ciprofloxacin, piperacillin-tazobactam, cefoperazone sulbactam antibiotics.
- Of all cases (3) of Pseudomonas auriginosa, one case (33%) was resistant to cefotaxime, amikacin, ciprofloxacin, piperacillin-tazobactam, cefoperazone sulbactam antibiotics.
- Of two cases of Staphylococcus aureus, one case was (50%) resistant to cefotaxime, amikacin, ciprofloxacin, piperacillin-tazobactam, cefoperazone sulbactam antibiotics.
- Of two cases of Proteus mirabilis, all cases were sensitive to cefotaxime, piperacillin-tazobactam, cefoperazone-sulbactam antibiotics and one case (50%) was resistant to Ciprofloxacin and amikacin antibiotics.

All the isolated organisms were found to be sensitive to carbapenem antibiotic Meropenem which was used to change the antibiotic in treating the SBP patients once culture results are available.

Table:-1 Antibiotic sensitivity patterns in culture positive SBP cases

Organism	E.coli (n=13)		Klebsiella pneum(n=4)		Pseudomonas auriginosa(n=3)		Staphylococcus aureus(n=2)		Proteus mirabilis(n=2)	
Antibiotic	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Cefotaxime	9	4	3	1	2	1	1	1	2	0
Ciprofloxacin	8	5	3	1	2	1	1	1	1	1
Amikacin	9	4	3	1	2	1	1	1	1	1
Piperacillin +Tazobactam	11	2	3	1	2	1	1	1	2	0
Cefoperazone +Sulbactam	10	3	3	1	2	1	1	1	2	0
Meropenem	13	0	4	0	3	0	2	0	2	0

DISCUSSION

Correia and Harold Conn coined the term *spontaneous bacterial peritonitis* in 1975, to distinguish this form of infection from surgical peritonitis. The liver disease usually is chronic (cirrhosis), but may be acute (fulminant hepatic failure) or subacute (alcoholic hepatitis). Ascites is primarily a transudative fluid with a poor opsonic activity which provides a favorable environment for the growth of bacteria Ascitic fluid infection classification was proposed in 1998 .

1. Spontaneous ascitic fluid infection, which has three subtypes:

a) **Classical SBP**, diagnosed by an elevated ascitic fluid polymorphonuclear leukocyte (PMN) count >250 cells/mm3 and a positive ascitic fluid bacterial culture.

b) **Culture-negative neutrocytic ascites (CNNA),** is diagnosed by elevated ascitic fluid PMNL count >250 cells/mm3 with a negative ascitic fluid culture in the absence of even a single dose of antibiotic[1].

c) **Monomicrobial non-neutrocytic bacterascites (MNB)** includes a positive ascitic fluid culture for a single organism and ascitic fluid PMNL count <250 cells/mm3.

2. Secondary bacterial peritonitis:

Diagnosis of Secondary bacterial peritonitis is done when the ascitic fluid PMN counts are ≥ 250 Cells/mm3, culture showing polymicrobial organisms and an identifiable surgically treatable intraabdominal primary source of infection. The infection can occur with or without intestinal perforation.

3. Polymicrobial bacterascites:

Diagnosed when the PMN counts are < 250 cells/mm3, and the ascitic fluid shows cultures of multiple organisms. It is a rare event seen in about 1 in 1000 paracentesis occurring due to inadvertent perforation of the intestines while performing paracentesis.

PATHOGENESIS:

Current evidence suggests that the spontaneous ascitic fluid infections are due to translocation of the bacteria from the intestine to the mesenteric lymph nodes which results in spontaneous bacteremia and subsequent colonization of ascitic fluid[2].

ASCITIC – PERITONEAL HOST DEFENCE:

In healthy individuals, an efficient peritoneal defense mechanism clears off the entering organisms. But due to deficiencies in local defense mechanisms against bacteria in cirrhosis, the clearance of peritoneal bacteria is limited. The resident macrophages attract PMN by releasing chemotactic factors and also by activating the complement factors. One of the most potent chemokines identified is the Monocyte Chemotactic protein 1.Opsonic and bactericidal activity is reduced in patients with cirrhosis. Low opsonic activity is associated with a low C3 level and low total protein content. A C3 level of < 13 mg /dl is associated with ascitic fluid infection. With protein levels of < 1.5 mg/dl of ascitic fluid, the risk of SBP increases paralleling the decrease in protein content and the incidence rate increases to 27 – 44% at levels < 1g /dl[3].

GENETICS:

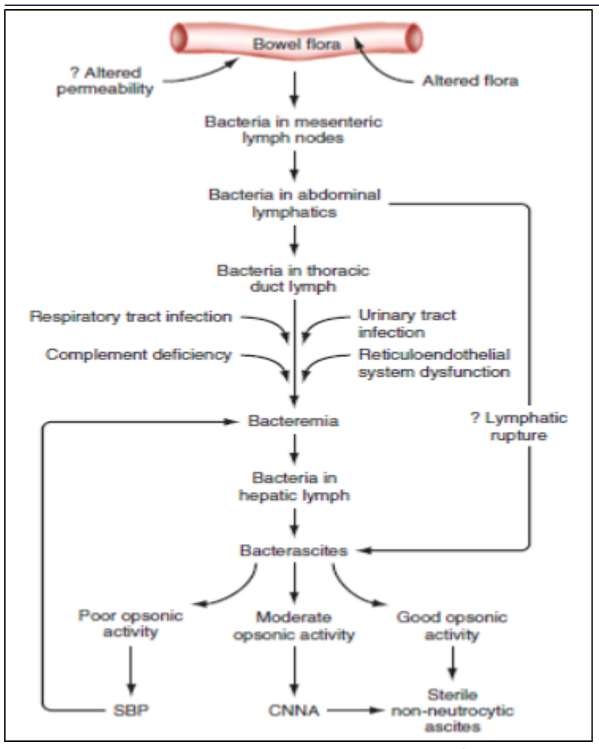
Persons with genetic variants such as CARD 15/NOD 2 and TLR2[4] polymorphisms have been found associated with an increased probability of acquiring SBP. Toll-like receptor 2 (TLR2) proteins are expressed in macrophages and are essential for the recognition of microbial components and host cell defense.

LIVER DYSFUNCTION:

Markers of advanced liver dysfunction have been identified as important risk factors for the development of SBP in a study done in 2007[5].

These include:

1) Bilirubin level greater than 3.2 mg/dl
2) Platelet count of less than 98,000 /mm3.
3) Each point of MELD increases the risk of SBP by about 11%.



PREDISPOSING FACTORS FOR SBP INCLUDE[6]:

Child-Pugh Class C
Ascitic fluid protein < 1g /dl
Ascitic fluid C3 levels < 13 mg / dl
Gastrointestinal bleeding
Urinary tract infection
Iatrogenic factors: urinary bladder and intravascular catheterization
Previous episodes of SBP.

MICROBIOLOGICAL PROFILE AND DRUG RESISTANCE:

The emergence of antibiotic resistance and changing the profile to SBP causing bacteria has made standard treatment less reliable in some instances[7]. 8–22% of Enterobacteriaceae have cephalosporin resistance. Patients with prophylactic or therapeutic antibiotic treatment, nosocomially acquired SBP can have multidrug resistant organisms with increased morbidity, mortality. A more effective first-line empirical antibiotic treatment with a broader spectrum drug like Carbapenems should be employed in these patients. The reasons for this is due to increasing incidence of extended spectrum β – lactamase (ESBL) producing bacteria as well as multiresistant gram-positive bacteria such as Enterococcus faecium or methicillin-resistant Staphylococcus aureus (MRSA).

ESBL's leads to resistance to various antibiotics including third generation Cephalosporins and Monobactams, and also carry genes encoding resistance to antibiotics like Quinolones, Tetracyclines, and antifolates.

A study conducted by Bhat et al[8]. found that 11.6% of the total suspected cases had spontaneous bacterial peritonitis, out of which 57.1% were culture-negative neutrocytic ascites (CNNA), 35.8 % were classical spontaneous bacterial peritonitis and 7% monomicrobial non-neutrocytic bacterascites (MNB). Gram-negative bacilli (Klebsiella and E. coli) were the commonest organisms. The overall response rate to ceftriaxone was 62.8%. Among culture-positive patients, sensitivity rates to ceftriaxone were 50%, while it was 53.3% for quinolones, 70% for piperacillin-tazobactam, and 93.3% for cefoperazone– sulbactam combination. Thirty-day mortality was lower for CNNA compared to SBP (20% vs. 40%, $p < 0.001$) and for patients with response compared to no response to first antibiotic (11.3% vs. 53.8%, $p < 0.001$).

Badawy et al found that empirical treatment with cefotaxime is effective only in 81% of cases and meropenem is effective in cefotaxime-resistant cases. The isolated organisms in cefotaxime resistant group include enterococci, Acinetobacter, expanded-spectrum β-lactamase producing Escherichia coli, β-lactamase producing Enterobacter and Staphylococcus aureus. A study by sheriff et al. has shown that SBP is responsive to third-generation cephalosporins in only 46% of cases and remaining cases require a change of antibiotic.

A study by Purohit et al[9] from India showed SBP in 43.8% of the clinically suspected cases. Among this 43.6% were culture positive and 56.4% were culture-negative neutrocytic ascites. From culture-positive cases, E.coli has isolated in 54.9% cases; Klebsiella spp.16.2% cases; Staphylococcus aureus 19.3% cases; and Pseudomonas aeruginosa was isolated from 9.6% cases. All isolates were sensitive to cefotaxime and ceftriaxone.

Table-2: Ascitic Fluid Culture Positivity : Comparision Table

Study series	Culture method used	Common organisms	Prevalance of SBP
Jeevan et al[53]	Bactec blood culture method at bed side inoculation	E.coli.(37.5%), Klebsiella(16.6%) Enterococcus (16.6%)	15.7%
Purohit et al[52]	direct inoculation of blood culture bottles at the bedside	E. coli (54.9%) Klebsiella (16.2%) Staphylococcus aureus (19.3%) Pseudomonas aeruginosa (9.6%) Culture positive in 43.6% cases	43.8%
Amarapurkar et al[77] (Used PMN >500/mm3).	1.Conventional Culture methods 2.Blood cultures	not detected in any cases detected in 60% cases	22%
Puri et al[79]	direct inoculation of blood culture bottles at the bedside	Culture positive in 62%	30%
Llach et al[18]	Bed side inoculation in blood culture bottles	E. coli (54.9%) Klebsiella (16.2%) Staphylococcus aureus (19.3%) Pseudomonas aeruginosa (9.6%)	43.8%
Castellote J et al[100]	Agar plates Blood culture bottles	Detected 57% episodes Detedcted 77% episodes	Studied in diagnosed cases of SBP by PMN count > 250/mm^3
Runyon BA[8]	Agar plates Blood culture bottles	Detected 43% episodes Detected 93% episodes	Studied in diagnosed cases of SBP by PMN count > 250/mm^3

PRIMARY PROPHYLAXIS:

The emergence of resistant bacteria is a problem encountered in long term prophylaxis. So, prophylactic antibiotics are indicated only in patients with the highest risk of developing SBP.
Prophylaxis is considered in the following settings[10]:

1. patients with ascitic fluid protein concentration <1.5 mg /dl,
2. variceal hemorrhage
3. child –Pugh score of ≥ 9
4. total bilirubin ≥ 3 mg/dl
5. serum creatinine of ≥ 1.2 mg /dl, blood urea nitrogen of ≥ 25 mg/dl
6. previous episode of SBP

In the setting of upper GI hemorrhage, Norfloxacin 400 mg twice daily for seven days is recommended to prevent SBP. Recently IV Ceftriaxone 1g daily for seven days is found to be more effective than Norfloxacin in the setting of GI hemorrhage, and in patients with advanced cirrhosis, i.e. with at least two of the following: ascites, severe malnutrition, encephalopathy or bilirubin >3 mg /dl.

SECONDARY PROPHYLAXIS:

In patients with the previous episode of SBP, Norfloxacin 400 mg orally once daily has to be given until death or liver transplantation. Some guidelines recommend the use of oral Ciprofloxacin 750 mg once weekly or Trimethoprim / Sulfamethoxazole in the dose of one double-strength tablet daily as an alternative.

CONCLUSION

The following conclusions could be done drawn from our study which consisted of 200 patients of decompensated liver disease in whom SBP prevalence was studied by ascitic fluid analysis and culture. In this study we identified E.coli being the most common organism for SBP followed by Klebsiella, Pseudomonas, Proteus, Staphylococcus. There is an increasing trend in the incidence of resistant organisms to empirical antibiotic like cefotaxime. 33% of E.coli were resistant to cefotaxime in our study, hence inappropriate antibiotic administration in the community for trivial reasons should be discouraged.

Antibiotic policy : Choosing empirical antibiotic for suspected cases of SBP should balance the inappropriate use of higher antibiotic and at the same time effective against probable organism. Empirical antibiotic should be administered after collecting the blood and ascitic fluid samples for analysis.

- Cefotaxime or Cefoperazone Sulbactam to be choosen as empirical antibiotic in suspected cases of SBP without high risk factors (advanced liver disease, upper gastrointestinal hemorrhage, hepatic encephalopathy and renal dysfucntion) as they are effective in 70 to 80% cases of most common organisms causing SBP.

- Piperacillin and Tazobactam to be choosen as empirical antibiotic in suspected cases of SBP with high risk factors for targeting the infectious agents as they are effective in 85% cases of common organisms causing SBP.

After obtaining culture results of ascitic fluid empirical antibiotic can be continued provided the organism isolated is sensitive to the administering antibiotic, otherwise to be changed to the sensitive antibiotic.

Routine ascitic fluid culture and appropriate antibiotic therapy is advisable to reduce the mortality and morbidity asscociated with SBP in decompensated cirrhosis patients.

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