Diagnostic Value of Leucocyte Esterase Reagent Strip Test in Cirrhotic Patients with Ascites for Early Detection of Spontaneous Bacterial Peritonitis

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Introduction

The word Ascites is derived from a Greek word “askos” meaning bag or sack. Ascites is the accumulation of excessive fluid within the peritoneal cavity.

Infection of Ascitic fluid can lead to 5 types of bacterial peritonitis-

(Sleisenger & Friedman Gastrointestinal and liver disease) 1

Spontaneous Ascitic fluid infection-

When there is no intra abdominal surgically treatable source of infection. It is of 3 types:
1. Spontaneous Bacterial Peritonitis.
3. Culture negative neutrocytic ascites.
4. Secondary Bacterial Peritonitis – when intra – abdominal surgically treatable cause is identified.
5. Iatrogenic (Polymicrobial bacterascites) – Infection induced during paracentesis.

Spontaneous Bacterial Peritonitis (SBP) is the infection of previously sterile ascitic fluid with no evidence of intra-abdominal surgically treatable cause of infection. Diagnosis is made when bacteria growth is seen in ascitic fluid culture and polymorphonuclear (PMN) count is greater than or equal to 250 cells/mm³.

Monomicrobial non-neutrocytic bacterascites (MNB) is diagnosis by positive ascitic fluid culture for a single organism and an ascitic fluid PMN count of less 250 cell/mm³.

Culture negative neutrocytic ascites (CNNA) is diagnosed when (1) ascitic fluid grows no bacteria (2) Ascitic fluid PMN count ≥ 250 cell/mm³. (3) without administration of antibiotics. (4) No other cause of elevation of PMN (eg. Haemorrhage into ascites).

The spontaneous variants of ascitic fluid infection (i.e. SBP, MNB and CNNA) occur only in the setting of severe liver disease. Although theoretically SBP may occur in any cases of ascites but practically it is presented almost exclusively by cirrhotics (due to any cause) with ascites.

Spontaneous Bacterial Peritonitis occurs in to 10 to 30 percent of patients of cirrhosis with ascites.

Most common bacteria causing SBP are organism of enteric origin. They may be gram negative aerobes like E. coli and Klebsiella or anaerobic organism. Organisms of non-enteric origin may include positive bacteria like pneumococcus and streptococcus viridians.

Micro-organisms pass from bowel to liver via the portal vein. From there, due to defective liver reticuloendothelial filter system, they gain an entry into the systemic circulation and this may cause infection of ascitic fluid.

Infection may occur due to translocation of bacteria across small intestinal wall via intestinal lymphatics. Infection may also occur due to transient bacteremia following local infections like respiratory or urinary tract infection.

It is important to diagnose SBP as early as possible because it is associated with increased incidence (30% as compared to 8% in patients without SBP) of hepatorenal syndrome type I (Vicente Arroyo, Monica Guevara, Pere Gines, et al 2002) 2 which has a mortality of 80% and a median survival of 14 days.

Leucocyte esterase reagent (LER) strips

Leucocyte esterase reagent (LER) strips, developed initially to test for PMNL in urine, have shown to be useful in detecting PMNL in other body fluids such as pleural fluid, cerebrospinal fluid and ascitic fluid. In studies in developed countries, this test reduced the time for diagnosis of SBP from few hours to a few minutes (Castellote J, López C et al 2003) 3
Leucocyte esterases catalyse the hydrolysis of derivatized pyrrole amino acid ester to liberate 3-hyroxy 5-phenyl pyrrole. This pyrrole subsequently reacts with a diazonium salt to produce purple product. This color change is read against a standard color chart provided with the reagent strips.

Multistix 10 SG are multiple reagent strips for analysis of urine. They detect leucocytes indirectly by detecting presence of leucocyte esterase enzyme activity in urine. They can be used to test ascitic fluid for the presence of leucocytes. Thus, strips can provide us a tool for rapid bedside diagnosis of SBP.

In developing countries like India with a large rural population, the need for a sensitive and rapid bedside diagnostic test for SBP is even higher. The present study was done to determine the accuracy and thus use of reagent strip in easily bedside diagnosis of SBP.

**Aim & Objectives**
1. To study leucocyte count by leucocyte esterase activity in ascitic fluid using reagent strips (multistix 10 SG) in patients of cirrhotic ascites.
2. To study cell type and count in ascitic fluid in patients of cirrhotic ascites.
3. To study presence of infection by ascitic fluid culture in patients of cirrhotic ascites.
4. To compare findings of leucocyte esterase reagent strip test, total neutrophil count & ascitic fluid culture in all the patients of cirrhotic ascites.

**Material and Methods**

The present study was conducted in Department of medicine, JLN Medical Collage and Associated Group of Hospitals, Ajmer. The subjects for study were taken from the patients attending medical outdoor and admitted in various medical wards. The present study included 160 patients of cirrhotic ascites regardless of etiology, age, sex, religion and ethnic group of patients.

**Exclusion Criteria**

Following patients were excluded from the study:
1. Patients with an identified, surgically treatable, intra-abdominal source infection viz. rupture viscera.
2. Haemodynamically unstable patients.
4. Pregnant patient.
5. Patients who refused paracentesis.
6. Immunocompromised state specially HIV.
7. Patient on steroid or immunosuppressive drug.

After admission, thorough clinical examination was carried out and data were recorded as per enclose proforma within 24 hours of admission and before any antibiotics were given. All subjects underwent abdominal paracentesis under strict conditions using a 20 gauge needle.

After assuring the patient, paracentesis was performed in the left lower abdominal quadrant by using aseptic technique. 50 cc of ascites fluid was drawn.

**Results**

**Table 1: Incidence of Spontaneous Bacterial Peritonitis Among Patients of Cirrhotic Ascites**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of cases</td>
<td>160</td>
</tr>
<tr>
<td>Patients with SBP</td>
<td>28(17.5%)</td>
</tr>
<tr>
<td>Patients without SBP</td>
<td>132(82.5%)</td>
</tr>
</tbody>
</table>
### Table 2: Liver Function Tests in Subject Studied

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Protein (gm/dl) (Mean ± SD)</th>
<th>Serum Albumin (gm/dl) (Mean ± SD)</th>
<th>Serum Globulin (gm/dl) (Mean ± SD)</th>
<th>SGOT (IU) (Mean ± SD)</th>
<th>SGPT (IU) (Mean ± SD)</th>
<th>S. Bilirubin (mg/dl) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhotic ascites without SBP (n=132)</td>
<td>6.13±0.39</td>
<td>3.19±0.30</td>
<td>2.95±0.28</td>
<td>101.14±44.98</td>
<td>71.62±40.72</td>
<td>3.18±1.68</td>
</tr>
<tr>
<td>Cirrhotic ascites with SBP (n=28)</td>
<td>6.09±0.47</td>
<td>3.21±0.40</td>
<td>2.81±0.26</td>
<td>155.71±78.29</td>
<td>117.14±84.37</td>
<td>3.52±0.92</td>
</tr>
<tr>
<td>Overall (n=160)</td>
<td>6.12±0.40</td>
<td>3.19±0.31</td>
<td>2.92±0.28</td>
<td>110.69±56.04</td>
<td>79.59±53.62</td>
<td>3.24±1.58</td>
</tr>
</tbody>
</table>

### Table 3: Ascites Fluid Total Protein in Subject Studied

<table>
<thead>
<tr>
<th>Group</th>
<th>Ascites fluid protein (gm/dl) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhotic without SBP (n=132)</td>
<td>2.03±0.40</td>
</tr>
<tr>
<td>Cirrhotic with SBP (n=28)</td>
<td>1.27±0.50</td>
</tr>
</tbody>
</table>

P value (Statistical Significance). Cirrhotic with SBP vs Cirrhotic without SBP <0.05 (HS)

### Table 4: Correlation Between SBP and Development of Hepatorenal Syndrome

<table>
<thead>
<tr>
<th>Group</th>
<th>Developed HRs</th>
<th>No HRs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhotic without SBP (n=132)</td>
<td>12(9.09%)</td>
<td>120(90.91%)</td>
<td>132</td>
</tr>
<tr>
<td>Cirrhotic with SBP (n=28)</td>
<td>8(28.6%)</td>
<td>20(71.4%)</td>
<td>28</td>
</tr>
</tbody>
</table>

P value <0.05 (Significant)

### Table 5: Correlation Between Development of SBP and Prognosis in Subjects Studied

<table>
<thead>
<tr>
<th>Group</th>
<th>Survivors</th>
<th>Non survivors</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhotic without SBP (n=132)</td>
<td>118(89.39%)</td>
<td>14(10.60%)</td>
<td>132</td>
</tr>
<tr>
<td>Cirrhotic with SBP (n=28)</td>
<td>20(71.4%)</td>
<td>8(28.6%)</td>
<td>28</td>
</tr>
</tbody>
</table>

P value <0.05 (Significant)

### Table 6: Comparison Of Polymorphonuclear Count Results By Microscopy And Reagent Strips

<table>
<thead>
<tr>
<th>No. of cell/mm³</th>
<th>Microscopy</th>
<th>Strips</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥250 or positive (+3) with strip</td>
<td>28(17.5%)</td>
<td>26(16.25%)</td>
</tr>
<tr>
<td>≤250 or negative with strip</td>
<td>132(82.5%)</td>
<td>134(83.75%)</td>
</tr>
</tbody>
</table>

Reagent strip test result

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>With SBP</th>
<th>Without SBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>132</td>
</tr>
</tbody>
</table>

The sensitive of regent strips was found to be 92.85%. The specificity of reagent was 100%. The positive predictive value was 100%. The negative value was 98.50%.

**Discussion**

The present study was conducted with the objective of finding the usefulness of leucocyte esterase reagent strip in early bedside diagnosis of spontaneous bacterial peritonitis. Total cell
count and cell type were found in the ascitic fluid. Culture of ascitic fluid was done in blood culture media and the results were compared with the result of reagents strips.

The study included 160 patients of ascites due to cirrhosis of liver regardless of the etiology of cirrhosis and their sex.

Initial diagnosis was based on thorough history and physical examination which was confirmed by appropriate investigations in each cases.

In our study, out of 160 patients studied, 132 (82.5%) were males and 28 (17.5%) were females. The mean age of males was 46.61±8.93 and that of females was 49.14±11.99. Overall mean age was 47.05±9.54 years. Maximum number of patients, 52 (32.5%), were in the age group 41-50 years.

K. Vidya Sagar et al (2016) did a study on cirrhotic patients with ascites. They found out that the mean age of cirrhotic ascites is 50.7 years which is similar to our study. In the study males were 86% and 14% of cirrhotics were females. Maximum number of patients (34%), were in the age group 40-49 years, which is also similar to our study.

Out of 160 cirrhotic patients studied, the cause was alcohol in 104 (65%) cases, post hepatitis B in 10 (6.28%) of the cases and cryptogenic and other in 46 (28.75%) of the cases.

The three most common causes of cirrhosis are alcohol, chronic hepatitis and NASH (cryptogenic) and this is consistent with the result of the present study. Sleisenger & Friedman (2015) of the cases.

Of the 160 patients studied 28 (17.5%) patients had spontaneous bacterial peritonitis. Most of them
were in the age group 31-40 (35.7%).

Caly WR, Strauss E et al (1993)5 suggested that the prevalence of SBP is 31% and Almdal TP, Skinhoj P et al (1987)6 in different studies suggested that the prevalence of SBP is 19% in the cases of cirrhotic ascites and the result are similar in present study.

The present study shows that out of 28 people with SBP, 6 (21.42%) were asymptomatic while fever (n=16, 57.14%) and pain abdomen (n=16, 57.14%) were the most common symptoms followed by hepatic encephalopathy (n=14, 50%).

Such J, Runyon BA (1998)7 in the study of patients of spontaneous bacterial peritonitis found out that fever is the most common presenting symptom(69%), abdominal pain(59%), hepatic encephalopathy(54%), paralytic ileus(30%). 10% were asymptomatic. Fever(68%), abdominal pain(49%), altered mental status(54%) are the most common presenting symptoms of SBP patients (Sleisenger and friedman vol 2 2015)3

In our study various liver function tests like serum bilirubin, total protein, albumin / globulin, SGOT and SGPT were studies in each patient. The mean value of serum bilirubin in patients without SBP was 3.18±1.68 while in those with SBP was 3.52±0.92. The mean value of SGOT and SGPT in patients without SBP were 101.14±44.98 and 71.62±40.72 while in patients of SBP these value were 155.71±79.29 and 117.14 ± 84.37 respectively. These result showed that liver functions were deranged in all the cases of cirrhotic ascites irrespective of the etiology or presence or absence of infection.

These result were consistent with the study of K.Vidya Sagar et al(2016)4 which showed that LFT was deranged more in patients with SBP than patients without SBP. Bilirubin, SGOT, SGPT were higher in cirrhotic ascites patients with SBP.

In our study, analysis of ascitic fluid showed that the mean cell count in patients without SBP was 28.72 ± 30.35 while in patient with SBP was 2141.0 ± 1469.5. The ascites fluid protein in patients without SBP was 2.03±0.40 while in patients with SBP, it was 1.27±0.50. This difference is high significant (P<0.05).

Runyon BA (1986)8 suggested that patients with low ascitic fluid protein are particularly susceptible to SBP. Thus, our results are comparable to this.

In our study, results of the growth on culture media showed that most common organism grown was E. coli (58.33%). Gram negative aerobes, i.e. bacteria of enteric origin were grown in 75% of the culture.

Mansour A. Parsi, Ashish Atreja, Nizar N. Zein (2004)9 in their article suggested that more than two third of all the cases of SBP were due to gram negative bacilli(50% of these being E. coli) and one fourth are due aerobic gram positive organism. The results of present study were comparable to the different studies.

In our study, out of the 132 patients without SBP, 12(9.09%) developed hepatorenal syndrome while out 28 patients with SBP, 8(28.6%) developed hepatorenal syndrome. Thus spontaneous bacteria peritonitis is associated with significantly increased incidence of HRS (P<0.05).

Vincent Arroyo, Monica Guevara and Pere Gines (2002)2 concluded that 30% of the cases of spontaneous bacteria peritonitis develop hepatorenal syndrome.

In our study, out of 132 patients without SBP 118(89.39%) survived while 14 (10.60%) died. The patients with SBP showed 28.6% mortality.

A study by Sonia Rito et al(2008)10 showed that mortality in SBP was 37%
A study by M. Poca et al (2016)\textsuperscript{11} showed that SBP patients treated with antibiotics and albumin had in-hospital mortality rate of 28%.

A study by Garcia Tsao G (2001)\textsuperscript{12} showed that even intensive treatment in the hospital, mortality in SBP is between 10% and 30%. These are similar to the present study.

In our study after comparing polymorphonuclear count result by microscopy with those by reagents strip it was found that the sensitivity of these strips was 92.58%, specific was 100%. The positive predictive value was 100% and negative predictive value was 98.50%.

Dr. Swathy Moorthy, Dr. S. R. Ramakrishnan et al (2015)\textsuperscript{13} showed that the sensitivity and specificity of LER strip when grade 3 (>125 PMN/cumm) was taken as cut off were 87.5% and 95.3% respectively with a positive predictive value of 80% and negative predictive value of 97.2%.The sensitivity and specificity of LER strip when grade 4 (>500PMN/cumm) was taken as cut off were 53.1%and 100% respectively with a positive predictive value of 100% and negative predictive value of 90.8%.

Summary And Conclusion

The present study was conducted on 160 patients of ascites due to cirrhosis of liver, regardless of the etiology of cirrhosis, at J.L.N. Medical College and Associated Group of Hospital, Ajmer.

The aim was to find out the usefulness of leucocyte esterase reagent strip in early bedside diagnosis of spontaneous bacteria peritonitis.

History, physical examination, detailed ascites fluid examination was done. Results of reagent strip were compared with that of microscopy.

Following observation were made in the present study:

1. Maximum numbers of patients were in the age group of 41 - 50 years.
2. Total number of males in the study were 132 and females were 28 giving a M : F ratio of 4.71 : 1.
3. Out of the total 160 patients 104(65%) were chronic alcoholics. Thus, alcohol was the most common cause of cirrhosis and consequently spontaneous bacteria peritonitis.
4. The incidence of spontaneous bacteria peritonitis in patients with cirrhotic ascites was 17.5%.
5. The most common presenting symptoms in patients with SBP were fever (n=16, 57.14%) and pain abdomen (n=16, 57.14%), followed by hepatic encephalopathy (n=14, 50%) decreased gastrointestinal motility (n=12, 42.85%) and gastrointestinal haemorrhage (35.71%). 21.42% (n=6) of the patients were asymptomatic.
6. Liver function tests were deranged in all the patients whether with SBP or without SBP.
7. The ascitic fluid cell count in patients without SBP was 28.72±30.35 while in patients with SBP, it was 2141.0±1469.5.
8. Ascitic fluid total protein was significantly lower in patients with SBP (1.27±0.50 vs 2.03±0.40 with p<0.05).
9. Aerobic gram negative bacteria were the most common (75%) organisms causing infection of ascitic fluid. E. Coli (58.33%) was the most common organism grown followed by Klebsiella pneumonia(16.66%, n=4), 14.2% of blood cultures showed no growth.
10. Spontaneous bacterial peritonitis is associated with an increased incidence of hepatorenal syndrome (28.6% as compared to 9.09% in patients without SBP with P value <0.05).
11. Spontaneous bacterial peritonitis is associated with a significantly increased mortality rate [28.6% as compared to 10.60% among patients without SBP (p<0.05)].
12. The sensitivity of leucocyte esterase reagent strips was found to be 92.85%, the specificity was 100%. The positive predictive value was 100% and the negative predictive value was 98.50%.

Conclusion

This study concludes that Leucocyte esterase reagent strip test at bedside is an important and cost-effective tool for diagnosis of Spontaneous bacterial peritonitis because early diagnosis of Spontaneous bacterial peritonitis may reduce the incidence of Hepatorenal syndrome and mortality.

REFERENCES

13. Dr. Swathy Moorthy, Dr. S. R. Ramakrishnan et al. Efficiency Assessment of Leucocyte Esterase Reagent strips in rapid bedside diagnosis of Spontaneous Bacterial Peritonitis - a comparison study with the gold standard Absolute Neutrophil Counts in ascitic fluid. Volume : 5 | Issue : 10 | October 2015 | ISSN - 2249.