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LEUKOCYTE ESTERASE IN ASCITES FLUID FOR DETECTING SPONTANEOUS BACTERIAL PERITONITIS IN LIVER CIRRHOSIS

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ABSTRACT

Spontaneous Bacterial Peritonitis (SBP) is a frequent complication in liver cirrhosis with ascites patients. Spontaneous bacterial peritonitis is often without symptoms, so diagnosis is often delayed. Ascites fluid analysis is expensive, while the ascites fluid culture, as the gold standard, takes a long time and is expensive too. Besides, not all hospitals have the facilities to do both tests. Dye strip test that detect leukocyte esterase, that was originally developed to detect the presence of polymorphonuclear cells in the urine was also sensitive and accurate for detecting the presence of polymorphonuclear cells in the ascites fluid. This examination is easy and quick to do and very cheap so that can be used for early detection of SBP. To determine the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of dye strip leukocyte esterase test for early detection of SBP in liver cirrhosis with ascites patients were studied. This study used a dye strip leukocyte esterase (Combur¹⁰ Test®M) in 28 samples of ascites fluid and compared with the results of ascites fluid culture. The ability of the leukocyte esterase test as a diagnostic test was very good at a cut-off +2 with 94.1% specificity, 63.6% sensitivity 32% PPV and 80% NPV. Examination leukocyte esterase in the ascites fluid could be used for early detection of SBP in liver cirrhosis with ascites patients and could help to exclude SBP.

Key words: Leukocyte esterase, early detection, liver cirrhosis, spontaneous bacterial peritonitis

INTRODUCTION

Liver cirrhosis is the end stage of various liver diseases characterized by fibrosis.^{1,6} Ascites is a pathological fluid accumulation in the peritoneal cavity as a frequent complication of liver cirrhosis. Approximately 10%-30% of cases of liver cirrhosis with ascites may progress to Spontaneous Bacterial Peritonitis (SBP).^{2,3} Spontaneous bacterial peritonitis is defined as a spontaneous infection of ascites fluid in the absence of an apparent source of intra-abdominal infection or inflammation.

Spontaneous bacterial peritonitis is a common complication in patients with liver cirrhosis due to late diagnosis. Portal hypertension causes a bacterial translocation in the intestine that cannot be eliminated due to immune system disorders in liver cirrhosis. Abdominal pain and fever are the most common symptoms of SBP, followed by vomiting, ileus, diarrhea, hepatic encephalopathy, gastrointestinal bleeding, and kidney failure. However, most patients with spontaneous bacterial peritonitis have no symptomatic clinical manifestation. Therefore, establishing the diagnosis of SBP is not sufficient only by exploring clinical symptoms but also by investigating ascites fluid analysis.

Polymorphonuclear cell (PMN) counting (≥ 250 cells/mm³) and positive culture of ascites fluid are the gold

standards to diagnose SBP, but both take a long time and are expensive. Also, not all hospitals have facilities to perform these examinations. Therefore, a rapid, easy and inexpensive examination is needed as a diagnostic test of SBP.^{2,4} Leukocyte esterase dye test initially developed to detect the presence of polymorphonuclear cells in the urine was also sensitive and accurate to detect the presence of polymorphonuclear cells in other body fluids such as pleural fluid, cerebrospinal fluid, peritoneal fluid and seminal fluid. Many studies in developing countries, including India, showed an accelerated diagnosis process of SBP from a few hours to a few minutes.²⁻⁴

The reagent strip detects leukocyte esterase found in granulocytes, monocytes, and macrophages. The principle of the dye test is based on the activity of leukocyte esterase which breaks the ester present in the reagent strip. The colorless indoxyl ester degradation by granulocytes becomes unstable and is easily oxidized to a violet color.^{5,6}

The high number of deaths caused by SBP up to 80% has prompted the researchers to examine whether the leukocyte esterase test could be used as an accurate diagnostic test and rapidly diagnose SBP in the Adam Malik Hospital Medan and other healthcare centers in areas with limited facilities.

METHODS

This study used a diagnostic test comparing sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of leukocyte esterase dye test examination to ascites fluid culture examination for diagnosing SBP. The study was conducted in the Department of Clinical Pathology, Faculty of Medicine, University of North Sumatra/Adam Malik Hospital Medan in collaboration with the Department of Internal Medicine Faculty of Medicine, University of North Sumatra. The study population comprised patients diagnosed with liver cirrhosis with ascites based on clinical symptoms and laboratory examination and willing to follow the study by signing informed consent.

RESULT AND DISCUSSION

The study, conducted in January 2016 to May 2016, collected 28 ascites fluid samples from liver cirrhosis patients with ascites.

Table 1 showed the characteristics of research samples in the form of age, and gender. Of the 28 samples examined there were 13 males (46.4%) and 15 females (53.6%). The most were in the age group of 42-50 years, as many as 11 patients (39.2%), followed by age group 60-70 years as many as seven patients (25%) and the lowest were in the age group 33-41 years as many as one patient (3.6%). The subjects in this study were mostly included in Child-Pugh C as many as 21 people (75%), while the remaining seven people (25%) were Child-Pugh B.

Table 1. Characteristics of study subjects by sex and age (n = 28)

Characteristics	N	%
Sex		
Males	13	46.4
Females	15	53.6
Age (years)		
23 – 32	5	17.8
33 – 41	1	3.6
42 – 50	11	39.3
51 – 59	4	14.3
60 – 70	7	25
Child-Pugh (class)		
B	7	25
C	21	75

Table 2 showed that of 28 fluid samples examined, 11 samples (39.3%) showed positive culture results with leukocyte esterase +1 test results in 4 samples, +2 in 5 samples and +3 in 2 samples. While 17 samples (61.7%) showed no growth results with negative leukocyte esterase count of 12 samples, +1 in 4 samples and +3 in 1 sample.

The types of bacteria that grew significantly varied from Gram (-), Gram (+), and fungi. Of the 11 types of bacteria that grew, 5 bacteria were Gram (-) group namely *Escherichia coli*, *Burkholderiacepacia*, *Salmonella sp.*, *Acinetobactercalcoaticus* and *Enterobacter cloacae*. While the 5 bacteria were Gram (+)

bacteria belonged to the species *Streptococcus sp.* and *Staphylococcus sp.* and 1 of them was fungi (Table 3).

Table 2. Results of the leukocyte esterase test connected with the presence or absence of bacterial growth

Leukocyte esterase	Bacterial growth	No bacterial growth
Negative	0	12
+1	4	4
+2	5	0
+3	3	1
Total	11(39.3%)	17(61.7%)

In Table 4, showed that the esterase leukocyte test had a good specificity with the cut-off value of +2 and +3 of 94.1%. The leukocyte esterase test had the highest sensitivity at the cut-off value of +1 that was 100%, while

Table 3. Microorganism patterns isolated from ascites fluid

Types of Microorganism	Total	Types of Microorganism	Total
Gram negative bacteria		Gram positive bacteria	
<i>Escherichia coli</i>	1	<i>Streptococcuspyogenes</i>	1
<i>Salmonella sp</i>	1	<i>Staphylococcus aureus</i>	1
<i>Enterobacter cloacae</i>	1	<i>Staphylococcus epidermidis</i>	2
<i>Acinetobactercalcoaticus</i>	1	Fungi	1
<i>Burkholderiacepacia</i>	1		

the cut-off value of +2 was 63.6%. The accuracy of the esterase leukocyte test in this study was 82%.

This study was a diagnostic test of leukocyte esterase test for early detection of SBP in patients with ascites liver cirrhosis by using ascites fluid culture as the gold standard. Comprising 28 ascites fluid samples from patients diagnosed with decompensated liver cirrhosis based on clinical symptoms and laboratory examination in the Department of Internal Medicine Adam Malik Hospital and who fulfilled the inclusion criteria. Ascites fluid samples were taken by paracentesis.

Characteristics of the 28 subjects who participated in the study consisted of 13 males and 15 females. The prevalence of liver cirrhosis in this study was different from Ramon’s data who disclosed that 60% of cases of liver cirrhosis were males in 2008. It was similar with data from the Dr. Sardjito Hospital in 2004 where male patients were more than females with a ratio of 1.5-2: 1 and data from Cipto the Mangunkusumo Hospital of 162 patients, where 94 patients were males and 68 females. The cause of this condition was liver cirrhosis patients who became the subject of this study and the ones who underwent the most paracentesis action were females. However, it did not describe the overall

Table 4. Performance characteristics of leukocyte esterase test by using 3 different cut-off scores

Variable	Cut-off value		
	+1	+2	+3
Sensitivity (%)	100	63.6	18.2
Specificity (%)	70.6	94.1	94.1
PPV (%)	68.7	35	66.7
NPV (%)	100	80	64
Accuracy (%)	82	82	82

The most significant number of liver cirrhosis patients in this study was in the age group 42-50 years as much as 39.3%. These results were not much different from the Cipto Mangunkusumo Hospital data where the most ages were between 31-50 years old.⁷

Patients with liver cirrhosis were classified by Child-Pugh classification in which 75% of the study subjects were included in Child-Pugh C and 25% were Child-Pugh B, whereas cirrhosis patients belonging to the Child-Pugh C classification had a risk of spontaneous bacterial peritonitis as much as 71%. The researchers used the dye of leukocyte esterase Combur¹⁰ Test®M. The cut-off +2 was the best with a 94.1% specificity and a 63% sensitivity from the results of the esterase leukocyte test associated with liquid culture as the gold standard. Although cut-off +1 had a sensitivity of 100%, its specificity was lower at 70.6%. The result was similar to a research conducted by Rungsun *et al.* whose the results showed the sensitivity level of 63% and 96% specificity with a cut-off +2.⁴ The results of this study differed from the results of research conducted by Susanna who found a sensitivity of 92.86% and specificity of 100%.⁸ Similarly, a study conducted by Thevenot *et al.*, in 2004 with the result of 100% specificity and 89% sensitivity and the results of a study by Campillo *et al.* with a 90.4% specificity and 80.4% sensitivity.⁹ This difference of results might be due to the difference in the number of research subjects, as in this study the number of subjects was less than the other studies.

Based on the pattern of microorganisms and antibiotic sensitivity patterns, it was found that five positive cultures of Gram-negative bacteria were the bacterium class *E.coli* which is the most common cause of SBP. Also, *Salmonella* was also found in this research. Both of these bacteria were suspected to be ESBL-producing bacteria (Extended Spectrum Beta-Lactamase). A bacteria was suspected of producing ESBL when it was resistant to at least 2 of the antibiotics ceftazidime, cefotaxime or ceftriaxone. In this study, the two bacteria were resistant to cefotaxime and ceftriaxone. Other Gram-negative bacteria encountered were *Acinetobacter calcoaceticus* which is a polymerized bacteria in humans, 42.5% in healthy humans and 75% in hospitalized patients. These bacteria caused nosocomial infections through intravenous catheters usage. *Enterobacter cloacae* and *Burkholderiacepacea* are often

isolated from the hospital environment and cause nosocomial infections. These bacteria are often resistant to many antibiotics.¹⁰ The researchers found that these three bacteria were still sensitive to amikacin antibiotics, ciprofloxacin and tazobactam, they also found *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* as Gram-positive bacteria. Most infection by *Staphylococcus epidermidis* are obtained in a hospital with predisposing factors such as catheters, implants and immunosuppressive therapy. *Streptococcus pyogenes* was sensitive to Vanomycin antibiotics. *Staphylococcus aureus* often causes nosocomial infection and have a resistance to vancomycin.¹¹ This is consistent with the results of the antimicrobial susceptibility pattern in this study. The most of the bacteria that could be isolated in this study was *Staphylococcus epidermidis*.

CONCLUSION AND SUGESTION

The results of this study showed the ability of reagent strips to detect leukocyte esterase in granulocyte cells in ascites fluid in patients with severe liver cirrhosis. The leukocyte esterase test was a very good diagnostic test with a specificity of 94.1%. A negative esterase leukocyte examination might help rule out the diagnosis of SBP with a positive predictive value of 80%. The pattern of bacteria obtained in this study consisted of 50% Gram-negative and Gram-positive of also 50%. The antimicrobial sensitivity pattern for Gram-positive bacteria in this study showed resistance to the first line drugs such as ciprofloxacin. Gram-negative bacteria showed resistance to the first-generation of cephalosporin antibiotics such as cefadroxil and third-generation cephalosporins such as cefotaxime and cefixime.

Leukocyte esterase tests can be used to detect SBP early in liver cirrhosis patients. Further research is needed with a larger number of subjects and using other brands of esterase leukocyte reagent strips to compare the specificity and sensitivity of these tests.

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