# EVALUATION OF LEUKOCYTE ESTERASE REAGENT STRIPS TEST IN THE DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS IN CHILDREN WITH CIRRHOSIS

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ABSTRACT - Background - Spontaneous bacterial peritonitis is defined as an ascetic fluid infection without an evident intra-abdominal surgically treatable source. Spontaneous bacterial peritonitis is one of the severe complications in patients with cirrhosis and ascites. Without early antibiotic treatment, this complication is associated with high mortality rate; therefore, early diagnosis and treatment of spontaneous bacterial peritonitis is necessary for survival. Leukocyte esterase reagent can rapidly diagnose the spontaneous bacterial peritonitis. Objective - This study aimed to find out the diagnostic accuracy of leukocyte esterase dipstick test for the diagnosis of spontaneous bacterial peritonitis. Methods - A single centered hospital-based cross-sectional study was conducted during July 2013 to August 2014 on children with cirrhotic liver disease and ascites who were admitted in the Department of Pediatric Gastroenterology in Nemazee Hospital affiliated to Shiraz University of Medical Sciences (Iran). All patients underwent abdominal paracentesis, and the ascitic fluid was processed for cell count, leukocyte esterase reagent strip test (Combiscreen SL10) and culture. Spontaneous bacterial peritonitis was defined as having a polymorphonuclear count (PMN ≥250/m³) in ascitic fluid. Sensitivity, specificity, positive predictive value and negative predictive value of leukocyte esterase test were calculated according to the formula. Results - Totally, 150 ascitic fluid sample of cirrhotic male patients (53.2%) and their mean age (4.33±1.88 years) were analyzed. Biliary atresia (n=44, 29.4%) and idiopathic neonatal hepatitis (n=29, 19.3%) were the most frequent etiology of cirrhosis. Also, abdominal pain (68.6%) and distension (64%) were the most common presenting complaint. Of all cases, 41 patients (27.35%) were diagnosed to have spontaneous bacterial peritonitis (PMN ≥250/mm³). Sensitivity and specificity of leukocyte esterase reagent test according to PMNs ≥250mm<sup>3</sup> were 87.80% and 91.74%, also on ascitic fluid culture results were 88.23% and 77.44%. Positive predictive value and negative predictive value of this test in PMNs ≥250mm³ were 80% and 95.23% and in cases with positive culture 33.33% and 98.09% were obtained, respectively. Efficiency of leukocyte esterase reagent test in diagnosing spontaneous bacterial peritonitis, according to PMNs ≥250mm³ and culture results were 90.66% and 78.66%. Conclusion - The leukocyte esterase strip test may be used as rapid test for diagnosis of spontaneous bacterial peritonitis due to its high diagnostic validity.

HEADINGS - Peritonitis. Ascites. Ascitic fluid. Neutrophils. Esterases. Leukocyte esterase. Child. Liver cirrhosis.

#### INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is one of the most common and severe complication of cirrhotic patients<sup>(5, 18,21)</sup>. Without early therapeutic intervention, mortality of SBP is about 20%-40% <sup>(22)</sup>. The characteristic of the SBP is spontaneous infection of the ascetic fluid without an intra-abdominal source of infection<sup>(10, 20, 25)</sup>. SBP patients usually have symptoms like fever, abdominal pain, impaired renal function, hypertension and progression to encephalopathy.

SBP prevalence declines about 30%-40% due to early diagnosis and treatment with broad-spectrum antibiotics<sup>(8, 11)</sup>. Despite impressive advances in medical care of patients with advanced liver disease in recent decades, SBP is still a common problem and cause of death of bacterial infection in these patients<sup>(1,24)</sup>. Most methods used in the diagnosis of SBP in the clinical practice are based on demonstration of polymorphonuclear (PMN) in the peritoneal fluid. Globally, irrespective of culture, the PMN ≥250/mm³ in ascitic fluid is known as the best marker of peritonitis<sup>(5, 14, 22)</sup>.

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However, the diagnosis of SBP takes hours by cell counter method. On the other hand, limitations in the adequate and necessary laboratory facilities and the long duration of ascitic fluid cultures are the causes of the delay in diagnosis of SBP with ascetic fluid cultures. This condition implies the necessity of a rapid diagnostic test. Leukocyte esterase reagent (LER) dipstick is a test that removes this time limit, such that the diagnosis of peritonitis is possible with this test within a few minutes<sup>(2, 17)</sup>. Recently, this test has been used for rapid diagnosis of infection in any type of body fluid, such as pleural fluid, ascetic fluid, and cerebrospinal fluid (CSF)<sup>(6, 17, 23)</sup>. The LER basic principle is interaction between leukocyte esterase and esterifies compound in the reagent strip yielding a violet azoic dye<sup>(15, 22)</sup>. Further studies in diagnosis of SBP using LER test have been performed on adults but a comprehensive study of the performance of this test has not been performed in children. Using this test, physicians may perform a timely diagnosis, clinical management and appropriate treatment. This study aimed to find out the diagnostic accuracy of leukocyte esterase dipstick test for the diagnosis of spontaneous bacterial peritonitis.

## Study design

A single centered hospital-based cross-sectional study was conducted during July 2013 to August 2014 on children with cirrhotic liver disease and ascites who were admitted in the Department of Pediatric Gastroenterology in Nemazee Hospital affiliated Shiraz University of Medical Sciences (Iran), the major referral center in the field of hepatology and liver transplantation in children in Iran. This study was approved by the Ethics Committee of the University and written consent was obtained from all parents after informing them about this study. All the parents were asked to sign the informed consent. Patients who had received antibiotics within the previous week within or outside the hospital for any reason, patients with a history of surgery for any reason in the past month, hematemesis, primary renal dysfunction, secondary bacterial peritonitis, peritonitis carcinomatosis, pancreatic ascites, subjects with portal hypertension due to malignancy or tuberculosis were also excluded.

Patients aged less than 19 years with cirrhosis of the liver, regardless of the gender, were selected as the study population. A total of 150 cirrhotic patients were included in the current study. Demographic and clinical information was collected through questionnaires, oral interview, and careful physical examination. Differential cell count and cytology were examined with a conventional optical microscope. The diagnosis of cirrhosis in our study was according to histological, clinical and sonographic findings of the liver. In this study, ascitic fluid PMN count ≥250/mm³ with or without a positive culture for ascetic fluid (without contiguous source of intra-abdominal infection) was considered as standard for diagnosis of SBP.

#### **Paracentesis**

All patients underwent abdominal paracentesis under aseptic condition. With the use of aseptic techniques, 20mL

of ascetic fluid was sent for routine biochemical and culture test. The reagent strip (Combiscreen SL10, Analyticon, Germany) was immersed in 5mL of ascetic fluid placed on a dry and clean container as described by the manufacturer for identification of LER test. After 2 minutes, the reagent strip was read comparing the color of the leukocyte reagent strip area with the colorimetric 5-grade scale depicted on the bottle. The test is based on the esterase activity of the granulocytes present in the ascetic fluid that reacts with an ester releasing 3-Hydroxy-5-phenyl-pyrrole. This reaction causes a color change in an azo dye (purple). Results of leukocyte esterase reagent strip were compared with ascetic fluid PMN cell count.

## Diagnostic criteria

The diagnosis of SBP was based on a PMN ≥250/mm<sup>3</sup> in ascitic fluid, irrespective of a positive ascetic fluid culture and clinical signs of SBP, and an absence of intra-abdominal sources of infection or inflammation<sup>(16)</sup>.

## Statistical analysis

Data are presented as means ±SD for quantitative variables and as frequencies for qualitative variables the exact 95% confidence interval for each statistic was calculated from the binominal distribution. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated according to their standard formulas. SPSS statistical software version 14.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

### RESULT

In total, the data of 150 patients were analyzed. Seventy-nine (52.3%) patients were male and 71 (43.7%) female (P=0.362). Mean  $\pm$ SD age of patients was 4.88 $\pm$ 4.37 years (range 1 month to 18 years). Mean age of females was 4.92 $\pm$ 3.49 years (range 2 months to 18 years) and that of males was 8.83  $\pm$  4.73 years (range 1 month to 16 years) (P=0.341).

The most common symptom in subjects was abdominal pain (68.6%), distension (64%) and fever (58.6%), respectively. The prevalence of symptoms of vomiting, irritability, loss of consensus and poor feeding was 29.3%, 23.3%, 13%, 3%, 26%, respectively. The most common etiology of patients was biliary tract artesia (27.6%) followed by idiopathic neonatal hepatitis (19.3%) and lowest prevalence of autoimmune hepatitis (2%) and congenital hepatic fibrosis (4.6%), (Table 1).

In our study, the positive and negative LER test separately based on number of PMNs and culture were evaluated. Positive and negative results of leukocyte esterase test in terms of the number of PMNs were shown in Table 2. According to PMN ≥250/mm³ of ascetic fluid, 41 (27.35%) patients were diagnosed with SBP (Table 2). Among them, leukocyte esterase test was negative in five patients (12.2%) and in 36 (87.8%) patients it was positive. Moreover, in 109 (72.6%) cases we obtained the PMNs <250mm³ with no diagnosis of SBP. Of them, 100 (91.8%) patients showed negative LER

TABLE 1. Characteristics of patients

Variable	
Sex (male/female, % )	79 (52.3%) / 71 (43.7%)
Age (mean ± SD, year)	$4.37 \pm 1.88$
Clinical symptoms	
Vomiting (%)	44 (29.3%)
Abdominal pain (%)	103 (68.6%)
Distension (%)	96 (64%)
Irritability (%)	35 (23.3%)
Loss of consciousness (%)	20 (13.3%)
Fever (%)	88 (58.6%)
Poor feeding (%)	39 (26%)
Etiology of cirrhosis (%)	
Idiopathic neonatal hepatitis (%)	29 (19.3%)
Biliary artesia (%)	44 (29.4%)
Cryptogenic cirrhosis (%)	8 (5.3%)
Wilson disease (%)	14 (9.3%)
Fulminant hepatitis (%)	12 (8%)
Tyrosinemia (%)	8 (5.3%)
Congenital hepatic fibrosis (%)	7 (4.7%)
Autoimmune hepatitis (%)	3 (2.0%)
Others (%)	25 (16.7%)

TABLE 2. Positive and negative result of LER test based on number of PMNs and culture

X7 - 11	LES test result		m 1
Variable	Negative	Positive	- Total
PMN count <250mm <sup>3</sup>	100 (91.8 %)	9 (8.2%)	109 (72.7%)
PMN ≥250/mm <sup>3</sup>	5 (12.2%)	36 (87.8%)	41 (27.3%)
Culture of fluid			
Negative	103 (77.5%)	2 (11.8%)	105 (70%)
Positive	30 (22.5%)	15 (88.2%)	45 (30%)

 $LER: leukocyte\ esterase\ reagent;\ PMN:\ polymorphonuclear;\ LES:\ leukocyte\ esterase.$ 

test result and 9 (8.2%) revealed a positive test result. Of the total of 150 patients, 105 (70%) had a negative test result and 45 (30%) had a positive leukocyte esterase (LES) test result.

Among the total patients (150 patients), ascitic fluid cultures in 17 (11.3%) were positive and in 133 (88.6%) patients were negative. Of the 17 patients with positive cultures, 2 (11.7%) patients and 15 (88.2%) patients were found to have positive and negative result tested for leukocyte esterase, respectively. Also, of the 133 patients with negative culture results, 103 (77.5%) patients and 30 (22.5%) patients were found to be positive and negative in leukocyte esterase, respectively. Sensitivity and specificity of LER test on the basis of PMN ≥250/mm³ were 87.80% and 91.74%.

Also, based on ascitic fluid culture results, sensitivity and specificity were obtained 88.23% and 77.44%, respectively. The PPV and NPV of this test based on the PMN ≥250/mm³ and positive cultures were obtained 80%, 95.23% and 33.33%, 98.09%, respectively. In total, accuracy of LER test in detecting SBP on PMN ≥250/mm³ was 90.66% and based on the culture results it was 78.66% (Table 3).

TABLE 3. Sensitivity, specificity, PPV, and NPV of the LES test in SBP

Variable	PMN ≥250/mm <sup>3</sup>	Culture positive
Sensitivity	% 89.80	% 87.00
Specificity	% 91.74	% 77.44
PPV	% 80	% 33.33
NPV	% 95.23	% 98.09
Accuracy	% 90.66	% 78.66

PPV: positive predictive value; NPV: negative predictive value; LES: leukocyte esterase; SBP: spontaneous bacterial peritonitis; PMN: polymorphonuclear.

## DISCUSSION

Spontaneous bacterial peritonitis is one of the important causes of mortality and morbidity of cirrhotic patients<sup>(19)</sup>. As the result, early diagnosis and treatment of these patients is essential in improving their clinical outcomes. Methods routinely used in the diagnosis of SPB have a time limit and a few hours to a few days are needed for diagnosis<sup>(20)</sup>. Also, measuring the number of ascitic PMN count is done by hematological methods using optical microscopy and manual counting. Although the PMN count of ascitic fluid is used as the gold standard for the diagnosis of SBP, this method is laborious and time-consuming. Spending a long time in diagnosis of SBP can cause an advancement in the stage of the disease and clinical implications. On the basis of these problems, considerable efforts have been made in recent years to develop an alternative test for a more rapid diagnosis of SBP. Leukocyte esterase enzyme has been shown as an important marker for PMN cell activity<sup>(3,9)</sup>. The efficacy of LER test for diagnosis of SBP has been reported in previous studies<sup>(4, 13, 18, 26, 28, 29)</sup>. Leukocyte esterase was used for urine analysis first, but recently it has been found to be useful across a wide range of body fluid infections<sup>(2, 17)</sup>.

This study was conducted to evaluate the accuracy of LES test for diagnosis of SBP in cirrhotic children. According to PMN ≥250/mm<sup>3</sup>, SBP was diagnosed in 41 (27.35%) patients. Of these, the leukocyte esterase test in five (12.1%) patients was negative and in 36 (87.8%) patients was positive. Also, in 109 (72.7%) patients PMNs <250mm<sup>3</sup> with no SBP; 100 (91.7%) patients had negative and 9 (8.2%) had a positive LER test result. Therefore in this study, sensitivity, specificity, PPV and NPV of LER test in diagnosing SBP were 95.23%, 80%, 91.74% and 87.80%, respectively. Also, based on ascitic fluid culture results, sensitivity, and specificity, PPV and NPV of LER test was obtained 88.23%, 77.44%, 33.33% and 98.09%, respectively. Although several studies have been conducted on the use of leukocyte esterase test in SBP, the results of these studies vary. In a study by Farahmand et al., nine patients with cirrhosis were studied and sensitivity, specificity, positive and negative predicate value for all of patients were derived 100% respectively<sup>(7)</sup>. Other studies have been done on adults, each reporting various reporting sensitivity and specificity. For example, in a study by Torun et al. performed on 63 consecutive adult patients with cirrhotic ascites, 15 subjects showed SBP. The sensitivity, specificity, PPV and NPV of the leukocyte esterase reagent strips were 93%, 100%, 100%, and 98%, respectively<sup>(27)</sup>. In another study done by Vanbiervliet et al. on 72 patients with cirrhosis, nine patients included were diagnosed with SBP, and LER test was positive in all cases with 100% sensitivity and specificity<sup>(28)</sup>. Butani et al. used LER test to diagnose SBP in 136 patients. The sensitivity, specificity, PPV and NPV value at this study were obtained 83%, 99%, 91%, and 98% respectively<sup>(3)</sup>. Following other studies on adults, Khatwani and colleagues reported the sensitivity, specificity, PPV, NPV of leukocyte esterase dipstick test to diagnose SBP 92%, 95%, 96%, and 90%, respectively in ninety four patients with cirrhosis<sup>(12)</sup>. Although several studies have been conducted on the use of LER test in SBP, the results of these studies are varied. Variation in the results of these studies can be partly explained by the use of different brands of reagent strips and the use of different cutoff points for positivity. The majority of studies have examined the use of leukocyte esterase testing in the diagnosis of peritonitis in adult. So far, only one study has been conducted by Farahmand et al. (7). using LER test in the diagnosis of SBP in children.

Our study is the first study carried out with a large sample size on the accuracy of LER test in diagnosing SBP in children. The mean age of the patients was 4.88 years (range 1 month to 18 years). As mentioned, in our study, accuracy of LER tests in detecting SPB was 90.66%. There is a great potential in using LER test for diagnosis of SBP in children. This test is easy, does not require expertise, is rapid, can be performed bedside with a low cost, and has high sensitivity and specificity for diagnosis of SBP. Also, saving time at diagnosis of SBP leads to rapid start of antibiotic therapy. LER test is also useful in determining the effectiveness of antibiotic therapy.

#### CONCLUSION

In conclusion, LER test strips is a very sensitive, specific and very rapid identification method for diagnosis of SBP in children with cirrhosis specifically. This is a rapid bedside test that is easy to use and can be performed efficiently in order to speed up the bedside diagnosis of this clinical entity. It fits easily into the work flow of a routine gastroenterology department and can be conducted in settings that do not have the facilities to carry out biochemical and bacteriological tests for diagnosis of SBP. In addition, a positive test result should be an indication for initiation of empirical treatment, and negative results should exclude SBP. Finally, LER test can be used extensively in paracentesis as a screening test to exclude ascitic fluid infection in children.

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## **Authors' contributions**

Honar N: main idea and supervisor of thesis. Geramizadeh B: laboratory analysis and quality control and supervisor of thesis. Dehghani SM: revision of manuscript and data collection. Kalvandi G: writing proposal and data collection. Shahramian I: data collection and revision of manuscript. Rahmani A: writing the draft and data analysis. Javaherizadeh H: literature search and revision of manuscript.

Honar N, Geramizadeh B, Dehghani SM, Kalvandi G, Shahramian I, Rahmani A, Javaherizadeh H. Avaliação de tiras reagentes de teste de esterase de leucócitos no diagnóstico de peritonite bacteriana espontânea em crianças com cirrose. Arq Gastroenterol. 2015,52(3):195-9.

RESUMO - Contexto - A peritonite bacteriana espontânea é definida como uma infecção do fluido ascítico sem evidente origem intra-abdominal cirurgicamente tratável. A peritonite bacteriana espontânea é uma das complicações graves em pacientes com cirrose e ascite. Sem tratamento antibiótico precoce, esta complicação é associada com alta taxa de mortalidade. Portanto, o diagnóstico precoce e tratamento de peritonite bacteriana espontânea são necessários para a sobrevivência. O reagente de esterase de leucócitos pode rapidamente diagnosticar a peritonite bacteriana espontânea. Objetivo - Este estudo teve como objetivo descobrir a acurácia diagnóstica do teste com tiras de esterase de leucócitos para o diagnóstico de peritonite bacteriana espontânea. Métodos - Um estudo transversal hospitalar unicêntrico foi realizado entre julho de 2013 e agosto de 2014 em crianças com cirrose hepática e ascite que foram admitidas no Departamento de Gastroenterologia Pediátrica no Hospital de Nemazee afiliado à Universidade de Ciencias Médicas de Shiraz (Irã). Todos os pacientes foram submetidos a paracentese abdominal, e o líquido ascítico foi processado para contagem de células, teste de tira de reagente de esterase de leucócitos (Combiscreen SL10) e cultura. Peritonite bacteriana espontânea foi definida como tendo uma contagem de polimorfonucleares (PMN ≥250/m³) no líquido ascítico. Sensibilidade, especificidade, valor preditivo positivo negativo do teste de esterase de leucócitos foram calculados de acordo com a fórmula. Resultados - Foram analisados um total de 150 amostras de líquido ascítico de pacientes cirróticos; (53,2%) eram do sexo masculino e sua média de idade (4,33±1,88 anos). A atresia biliar (n=44, 29,4%) e hepatite neonatal idiopática (n=29, 19,3%) foram as etiologias mais frequentes de cirrose. Além disso, dor abdominal (68,6%) e distensão (64%) foram as queixas mais comuns de apresentação. De todos os casos, 41 (27,35%) foram diagnosticados com peritonite bacteriana espontânea (PMN ≥250/mm³). A sensibilidade e especificidade do teste de reagente de esterase de leucócitos segundo PMN ≥250mm³ foi de 87,80% e 91,74% e, para os resultados de cultura de líquido ascítico, de 88,23% e 77,44%. Valor preditivo positivo e valor preditivo negativo do teste em PMN ≥250mm³ foi de 80% e 95,23% e em casos com cultura positiva 33,33% e 98,09%, respectivamente. A eficiência do teste de reagente esterase de leucócitos no diagnóstico de peritonite bacteriana espontânea, de acordo com resultados de ≥250mm³ e cultura PMN, foi de 90,66% e 78,66%. Conclusão - O teste de tiras de esterase de leucócitos pode ser usado como um teste rápido para diagnóstico de peritonite bacteriana espontânea, devido a sua alta validade diagnóstica.

DESCRITORES - Peritonite. Ascite. Líquido ascítico. Neutrófilos. Esterases. Leucócitos. Criança. Cirrose hepática.

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