Diagnosis of Spontaneous Bacterial Peritonitis in Children by Reagent Strips

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Abstract- This study was aimed to evaluate the efficacy of dipstick tests (leukocyte esterase and nitrite) in diagnosis of spontaneous bacterial peritonitis (SBP) in cirrhotic patients. Forty six children with ascites hospitalized between 2009 and 2010 in Children Medical Center were enrolled in this study. Reagent strip assays for leukocyte esterase and nitrite were performed on ascetic fluid and the results were compared to manual cell counting and ascitic fluid culture. SBP was defined as having a polymorphonuclear ascites count of $\geq 250/mm^3$. Twenty children were female and twenty six were male with mean age of 3±3.9 years. The sensitivity specificity, positive and negative predictive values of the leukocyte esterase reagent strips were all 100%. The sensitivity, specificity, positive and negative predictive value of the nitrite reagent strip test were 100%, 97%, 90% and 100% respectively. Leukocyte esterase reagent strips may provide a rapid, bedside diagnostic test for the diagnosis of SBP.

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Introduction

Spontaneous bacterial peritonitis (SBP), which is defined as ascitic fluid infection in the absence of an exact source, is a consequence of ascites in cirrhotic patients with prevalence rate of 3.5% to 30% (1-5). Fever, abdominal pain, nausea, and vomiting are common symptoms of SBP but they are not present in all children with SBP. Rapid and proper diagnosis of SBP can prevent death although mortality according to this complication is near 30-50% (6-9).

Routine diagnosis of SBP consists of ascitic fluid paracentesis and polymorphonuclear (PMN) leukocyte count more than 250 per mm^3 has been known as sensitive and practical method for diagnosis (10). Manual measurement of PMN count is not always the accessible way to identify SBP due to laboratory rush hours in referral hospitals or outpatient settings.

Leukocyte esterase is a sensitive and accurate test for detecting PMN cells in body fluids such as urine, cerebrospinal fluid (CSF), semen, pleural fluid and peritoneal fluid. Chemicals on the reagent strip reacts with esterase of the granulocytes present in the biological fluid and makes color changes that are visible on the strips (*e.g.* from red to purple) (11-13).

The aim of this study was to determine the sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) of nitrite and leukocyte esterase reagent test strips for the diagnosis of SBP in cirrhotic children with ascites.

Materials and Methods

This study was carried out at Children Medical Hospital (Tehran, Iran) between January 2010 and January 2011 and it was approved by the Ethics Committee of Tehran University of Medical Sciences. All parents asked to fill informed consent. Forty-six unselected cirrhotic Children who were hospitalized in Gastrointestinal ward were included and a total of 46 paracenteses were performed.

Cirrhosis was diagnosed based on histological criteria or clinical criteria (splenomegaly, ascites and/or esophageal varices) and ultrasonography findings. Paracentesis was performed for the ascites investigation. Ascitic fluid was examined for PMN and lymphocyte count as well as biochemical markers such as glucose, protein, albumin and lactic dehydrogenase. Differential cell count and cytology were examined with a conventional optical microscope. Ascetic fluid culture

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was carried out using MacConkey agar and chocolate agar. PMN count more than 250 per mm^3 with or without a positive culture for ascetic fluid (without contiguous source of intra-abdominal infection) was established as SBP diagnosis. After the paracentesis, the ascitic fluid was collected in a clean container then reagent strip for leukocyte esterase (Combur test® UX; Roche Diagnostics GmbH, D-68298 Mannheim, Germany) as well as nitrite strips were submerged into fluid and removed immediately. The strips were read by the physician who did the paracentesis. The strips had a colorimetric 4-grade scale (0-3). The correlation between PMN and the four-grade scale suggested by the manufacturer was as follows: grade 0: 0 PMN per mm^3; grade 1: 25 PMN per mm^3; grade 2: 75 PMN per mm^3; grade3: 500 PMN per mm^3. The reagent strips were considered positive if the grade was 2 or 3.

Data are presented as mean values and SDs. The sensitivity, specificity, positive predictive value and negative predictive value were determined.

Results

Forty six cirrhotic children with ascites were enrolled in this study. Twenty (43.4%) were female and twenty six (56.6%) were male and mean age of participants was 3 ± 3.9 years. SBP was diagnosed in nine patients (19.5%) by cytology (> 250 neutrophils per mm^3); however, all of these nine ascitic fluid cultures were positive (Four were positive for *Escherichia coli* and 5 for *Streptococcus pneumoniae*).

Reagent strip test for leukocyte esterase was positive in all of these patients while reagent strip test for nitrite was positive in ten. Fluid cultures were positive for *Streptococcus pneumoniae* in 5 children and 4 for *Escherichia coli*.

The sensitivity, specificity, PPV and NPV of the leukocyte esterase reagent strips were all 100% while the sensitivity, specificity, PPV and NPV of the nitrite reagent strips were 100%, 97.2%, 90%, and 100%, respectively.

Among the 9 patients with SBP, the leukocyte esterase strip tested 2+ in the ascitic fluid (who developed *Escherichia coli* in culture) of one patient and 3+ in the remaining 8 cases.

The etiology of cirrhosis is shown in Table 1. Cholestasis was most prevalent cause of cirrhosis. Cholestasis in our cases were mostly die to progressive familial intrahepatic cholestasis, Bile duct atresia, choledochal cysts, Giant cell hepatitis, cystic fibrosis, inflammatory bowel disease and hemophagocytic lymphohistiocytosis. Metabolic disorders (tyrosinemia, Gushe, Niemann pick 1, Galactosemia) were the cause of cirrhosis in 13 cases.

Table 1. Etiology of cirrhosis in patients.

Etiology	Number
Metabolic	13
Cholestasis	22
Cystic fibrosis	2
Inflammatory bowel disease	1
Hemophagocytic lymphohistiocytosis	1
Others	7

Discussion

To our knowledge this is the first study to evaluate accuracy of reagent strips for the diagnosing of SBP in cirrhotic children with ascites. As rapid diagnosis of SBP in cirrhotic patients is essential to avoid mortality and morbidity, accurate diagnostic tests for early diagnosis should be applied. Although ascitic fluid PMN count is the current method for diagnosis, this method takes time and may not be available in all emergent and outpatient settings.

Leukocyte esterase which has been used for urine analysis first, is widely applied for body fluid infections diagnosis as well as many studies confirmed its accuracy and validity for PMN cell detections (13). In our study sensitivity, specificity, PPV and NPV of the leukocyte esterase reagent strips which were used in adults were all 100%. Previous studies reported different test accuracies which shown in Table 2 (14-17).

Table 2. Literature review.

Author	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Castellote *et al.*	89	99	98	97
Butani *et al.*	89	99	89	99
Thévenot *et al.*	89	100	100	99
Rerknimitr *et al.*	88	81	55	96

These differences in validity of the test can be due to different commercial dipsticks with different colorimetric scales (17).

On the other hand, the sensitivity, specificity, PPV and NPV of the nitrite reagent strips were 100%, 97.2%, 90%, and 100%, respectively. In a study by Torun *et al,* 63 patients with cirrhosis ascites were evaluated by leukocyte esterase and nitrite reagent strip tests. They found sensitivity, specificity, PPV and NPV of the nitrite reagent as 13%, 93%, 40%, and 77% while these factors were 93%, 100%, 100%, and 98% for leukocyte esterase test (18). Previous studies suggest that nitrate concentration and nitric oxide levels raise in ascitic fluid in cirrhotic cases (19) although many factors may affect amount of nitrite and nitrate levels in body fluids such as infections, hepatic synthesis capacity and nitrate derived from food (20-22).

The incidence of SBP in our study was 19.5% which was lower than previously reported (82%) (10). The most grown bacteria was *Streptococcus pneumoniae* while in previous studies in adults the most frequent *Escherichia coli* (17,18).

In conclusion, we can conclude that reagent strips are rapid, feasible and low-cost tests with high sensitivity and specificity for diagnosing of SBP in cirrhotic children.

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