Research Article

Leucocyte Esterase Reagent (LER) Strips: a Rapid, Easy, and Cheap Test for the Diagnosis of Spontaneous Bacterial Peritonitis.

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Abstract
Background: Patients with cirrhosis and ascites are more susceptible to bacterial infections, of which spontaneous bacterial peritonitis (SBP) is the most frequent and potentially life threatening. Diagnosis should be prompt and treatment must not be delayed until the polymorph nuclear leukocyte (PMN) count & microbiology results are available. The aim of this study is to determine the diagnostic validity of leukocyte esterase dipstick test for the diagnosis of spontaneous bacterial peritonitis**.** **Methods:** This study included ١٠٠ patients with cirrhosis and ascites who attended Tropical Medicine Department & Internal Medicine Department, EL-Minia University Hospital from January ٢٠١٢ to June ٢٠١٢. Two samples of ascetic fluid for each patient were taken, One for polymorph nuclear leukocyte (PMN) count. The reagent strip (Multistix ١٠SGR, Bayer Diagnostics) was immersed in the other ascitic fluid sample. The results of the two samples were compared and the diagnostic validity were estimated. **Results:** Fifty-nine (٥٩٪) patients were diagnosed SBP by PMN cell count ≥٢٥٠/mm^{٣}. Fifty-six (٥٦٪) patients were diagnosed as SBP by leukocyte esterase dipstick test. Three (٣٪) patients' ascitic fluid PMN count was <٢٥٠/mm^{٣} while being positive by dipstick test. In ٥٣(٥٣٪) patients a dipstick reaction was positive and PMN cell count was ≥٢٥٠/mm^{٣}. On the other hand, thirty-eight (٣٨٪) patients' ascitic fluid were negative by dipstick test and PMN count was <٢٥٠/mm^{٣}, but dipstick test was negative in six (٦٪) patients with ascitic fluid while PMN cell count ≥٢٥٠/mm^{٣}. The sensitivity(sn), specificity(sp), positive predictive value (ppv), negative predictive value(npv) of leukocyte esterase dipstick test to diagnose SBP were ٨٩.٨%, ٩٢.٧%, ٩٤.٦٪, ٨٢% respectively. **Conclusion:** The leukocyte esterase dipstick test can be used as an easy, rapid, inexpensive bedside test for diagnosis of SBP due to its high diagnostic validity.
Keywords: Cirrhosis, Ascites, Spontaneous bacterial peritonitis, Validity and Leukocyte esterase.

Introduction
Cirrhosis is an immunocompromising state which predisposes the patient to a variety of infections[١]. Spontaneous bacterial peritonitis (SBP) is a serious complication of advanced cirrhosis of the liver. Usually patients with SBP show symptoms such as fever, abdominal pain, worsening of renal function, hypotension or development of encephalopathy. The frequency of SBP among hospitalised patients with advanced cirrhosis varies from ١٠٪ to ٣٠٪[٢]. The mortality of SBP was ٨٠٪ to ١٠٠٪ in the١٩٦٠s, but has declined to ٣٠٪ to ٤٠٪ with early diagnosis and effective therapy with broad-spectrum antibiotics[٣,٤]. Despite the advancement in medical care for patients with advanced liver disease in the past decades, bacterial infections remain very common and account for significant morbidity and mortality (approximately ٣٠٪) in these patients[٥,٦]. Today, an ascitic fluid polymorphonuclear (PMN) leukocyte count of ٢٥٠/mm^{٣}or over, irrespective of the (ascitic fluid) AF culture result, is universally accepted as the best surrogate marker for diagnosing SBP[٧].

Patients with SBP frequently relapse after the first episode, with recurrence in approximately ٩٠٪ of patients if untreated[٨]. Symptoms and signs are frequently absent in patients with SBP, so a diagnostic paracentesis should be perfo-rmed in all patients with ascites admitted to hospital regardless of whether or not there is a clinical suspicion. Diagnosis should be prompt

and treatment must not be delayed until the microbiology results are available[٩,١٠]. The ascitic fluid total leukocyte and PMN count are not always done stat, thereby delaying time to diagnosis. Therefore, a rapid, simple screening test is needed for the prompt diagnosis of SBP[١١].

Leucocyte esterase reagent (LER) strips, developed initially to test for PMNL in urine[١٢], have been shown to be useful in detecting PMNL in other body fluids such as pleural fluid[١٣,١٤], cerebrospinal fluid[١٥], and AF[١٦-١٨]. This test reduced the time for diagnosis of SBP from a few hours to a few minutes[١٧]. In this test, esterase activity of PMNL in the fluid acts on an ester substrate releasing ٣-hydroxy-٥-phenylpyrrole; this changes the colour of an azo dye in the reagent strip. This colour change is read against a standard colour chart provided with the reagent strips[١٥,١٦]. In the present study we aimed at determining the diagnostic accuracy of leukocyte esterase reagent strip test in the bedside diagnosis of spontaneous bacterial peritonitis in cirrhotic patients with ascites, so that rapid diagnosis and treatment can be established.

Subjects and Methods

The study was conducted in Tropical Medicine Department & Internal Medicine Department, Minia University Hospital from January ٢٠١٢ to June ٢٠١٢. One hundred Patients with cirrhosis and ascites of either gender were included in this study (Table ١). Patients with one or more of the following criteria were excluded from this study; Patients who received antibiotics within the previous week, patients with haematemsis, encephalopathy, deteriorated renal function, patients with secondary bacterial peritonitis, peritoneal tuberculosis, peritoneal carcinomatosis.

Those patients having secondary bacterial peritonitis due to surgical problem, patients with renal, cardiac or pericardial disease, patients with malignant ascites or hepatoma, all outdoor patients and those who were not consenting, were also excluded.

Detailed history and examination were performed and each patient was thoroughly investigated and tests like leukocyte count, liver function tests, prothrombin time, urea, creatinine and blood sugar were performed. Moreover, viral markers for every patient was done as a routine investigation. Abdominal ultrasound was done in all patients to evaluate liver size, surface and texture, splenic size and ascites. Purpose and procedure of paracentesis with its risks and benefits were discussed with patients and finally informed written consent was taken.

Applying full protocol of aseptic techniques, ١٠ ml of ascetic fluid was sent for routine biochemical and cytological tests. The reagent strip (Multistix ١٠ SGR, Bayer Diagnostics) was immersed in ٥mL of ascitic fluid placed on a dry and clean container as described by the manufacturer for identification of leukocyte esterase. After two minutes, the reagent strip was read comparing the colour of the leukocyte reagent strip area with the colorimetric ٥-grade scale depicted on the bottle. Based on the degree of colour change in the reagent strip area, the results were scored as negative, grade ١ or traces, grade ٢ or low, grade ٣ or moderate, and grade ٤ or high. The test is based on the esterase activity of granulocytes present in the ascitic fluid that reacts with an ester releasing ٣-Hydroxy-٥-phenyl-pyrrole.

This reaction causes a colour change in an azo dye (purple). All ascitic fluid samples were sent to the same laboratory for total and differential cell count. Results of leukocyte esterase reagent strip were compared with ascitic fluid PMN cell Count. The sensitivity, specificity, positive predictive value and negative predictive value calculated according to their standard formulas. Ascitic fluid PMN count ≥٢٥٠/mm^{٣} was taken as standard for the diagnosis of SBP.

Results

One hundred patients were enrolled in this study. Mean age of patients was ٤٦.٦±١٢.٤ years, ٦٩ (٦٩٪) patients were males and ٣١ (٣١٪) were females (Table ١). Two patients (٢%) were Child A, ٣٣(٣٣٪) were Child B, and ٦٥(٦٥%) were Child C (Table ١& Fig. ١). Results of hepatitis markers were ٢٢(٢٢%) HBsAg positive, ٥٦(٥٦%) Anti–HCVpositive, ١٧(١٧%) both HBsAg and Anti–HCV positive (Table ١). Fifty-nine (٥٩%) patients were diagnosed SBP by PMN cell count ≥٢٥٠/mm^{٣}. Fifty-six (٥٦%) patients were diagnosed as SBP by leukocyte esterase dipstick test. Three (٣٪)

patients' ascitic fluid PMN count were <٢٥٠/mm^{٣} and dipstick test positive. In ٥٣(٥٣٪) patients a dipstick reaction were positive and PMN cell count were ≥٢٥٠/mm^{٣} which is graded into, +١ positive in ٣(٥.٧%) patients, +٢ positive in ١١(٢٠.٧٪), +٣ positive in ٢٩(٥٤.٧%) patients, and + ٤ positive in ١٠(١٨.٩%) (fig.٢). Thirty-eight (٣٨٪) patients' ascitic fluid had negative dipstick test and PMN count was <٢٥٠/mm^{٣}, but dipstick test was negative in Six (٦٪) patients, with ascitic fluid PMN cell count was ≥٢٥٠/mm^{٣}.

According to leukocyte esterase dipstick test, True positive were ٥٣ (٥٣٪), true negative ٣٨ (٣٨٪), false positive ٣ (٣٪) and false negative were ٦ (٦٪) (Table ٢ & Fig. ٣).

The sensitivity, specificity, positive predictive value, negative predictive value of leukocyte esterase dipstick test to diagnose SBP were ٨٩.٨%, ٩٢.٧٪, ٩٤.٦%, ٨٢% respectively (Table ٢ & Fig. ٤).

Table (١): Patients characteristics

Characteristics	*Mean± SD or Number (%)*
Male sex	٦٩(٦٩٪)
Age (years)	٤٦.٦ ±١٢.٤
Child-Pugh score	
A	٢(٢٪)
B	٣٣(٣٣٪)
C	٦٥(٦٥٪)
Hepatitis markers	
HBsAg positive	٢٢(٢٢٪)
Anti HCV Positive	٥٦(٥٦٪)
Both HBsAg and anti HCV positive	١٧(١٧٪)

* SD: standard deviation.

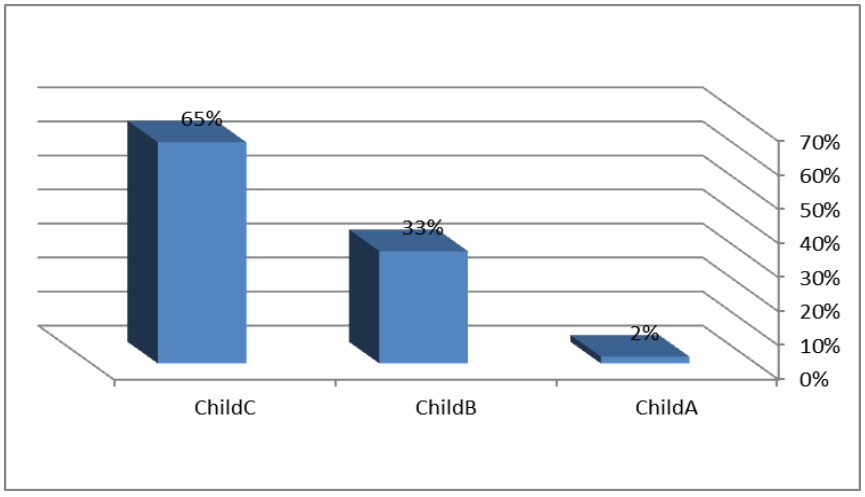

Fig. (١): Child Pugh grading of the studied patients.

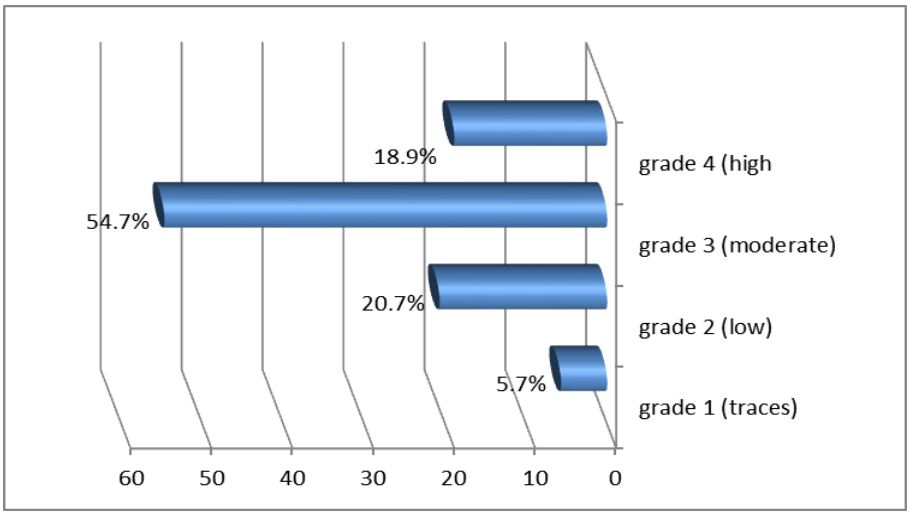

Fig. (٢): Grading of leukocyte esterase dipstick test of the studied patients.

Table (٢): Diagnostic validity of leukocyte esterase reagent strip in diagnosis of SBP.

True positive	٥٣(٥٣٪)
False positive	٣(٣٪)
True negative	٣٨(٣٨٪)
False negative	٦(٦٪)
Sensitivity	٨٩.٨٪
Specificity	٩٢.٧٪
Positive predictive value	٩٤.٦٪
Negative predictive value	٨٢٪

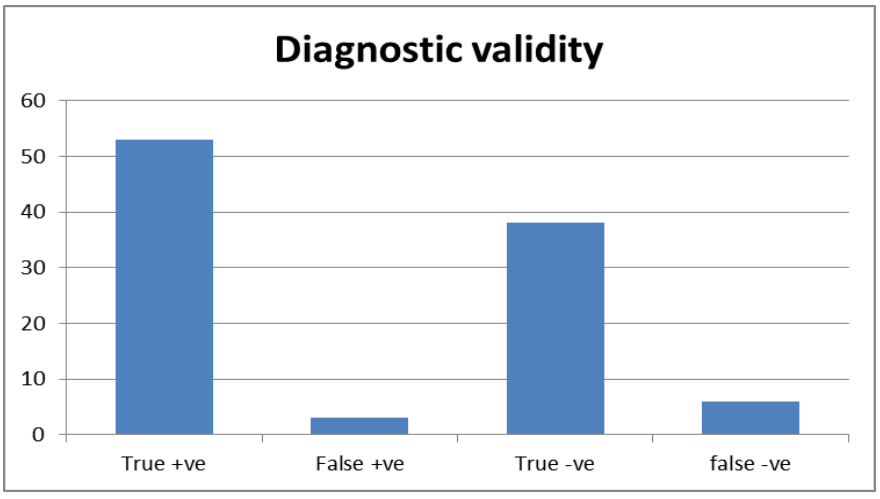

Fig. (٣): Diagnostic validity of leukocyte esterase reagent strip in diagnosis of SBP.

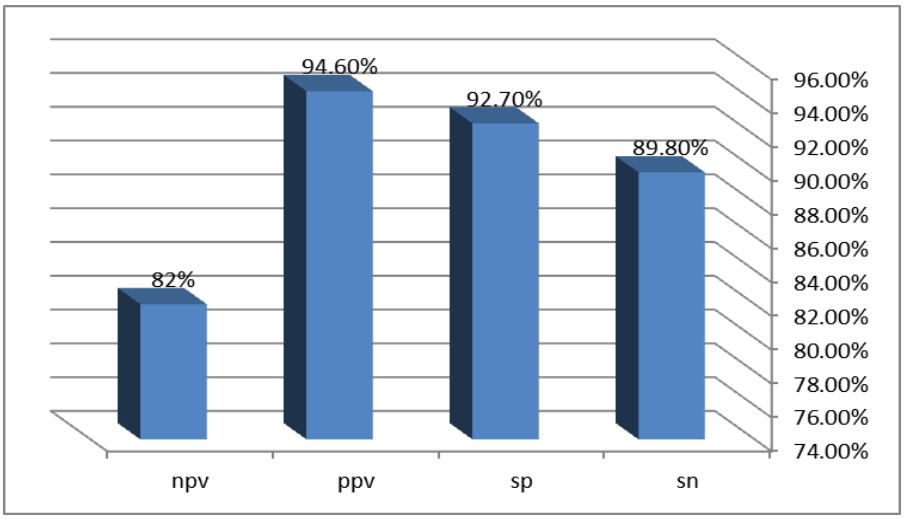

Fig. (٤): Diagnostic validity of leukocyte esterase reagent strip in diagnosis of SBP.

Discussion

Spontaneous bacterial peritonitis is the most frequent infection in patients with liver cirrhosis causing significant mortality[١٩].

Studies from ١٩٧٠ reported that prevalence of spontaneous bacterial peritonitis was ٥-١٠٪ in cirrhotic patients with ascites. Recently the prevalence of spontaneous bacterial peritonitis in cirrhotic patients with ascites admitted to hospital has been estimated at ١٠-٣٠٪[٢٠].

Spontaneous bacterial peritonitis (SBP) requires rapid diagnosis and the initiation of antibiotics. Diagnosis of SBP is usually based on cyto-bacteriological examination of ascitic fluid. These tests require good laboratory facilities and reporting time of few hours to ١–٢ day[٢١].

Its mortality has been decreased from ٨٠ to ٣٠٪ due to prompt diagnosis and early initiation of adequate treatment[٢٢]. Leukocyte esterase enzyme has been shown as an important marker for PMN cell activity[٢٣]. The efficacy of leukocyte esterase dipstick test for diagnosis of SBP has been established from many studies done at different centres[٢٤-٣٢]. The present study confirmed the high accuracy of leukocyte esterase dipstick test for rapid diagnosis of spontaneous bacterial peritonitis in cirrhotic patients with ascites.

In our study, Fifty-nine (٥٩٪) patients was diagnosed SBP by PMN cell count ≥٢٥٠/mm^{٣}. Fifty-six (٥٦٪) patients were diagnosed as SBP by leukocyte esterase dipstick test. Three (٣٪) patients' ascitic fluid PMN count was <٢٥٠/mm^{٣} and dipstick test positive. In ٥٣(٥٣٪) patients a dipstick reaction was positive and PMN cell count was ≥٢٥٠/mm^{٣} which is graded into, +١ positive in ٣ (٥.٧٪) patients, +٢ positive in ١١(٢٠.٧٪), +٣ positive in ٢٩ (٥٤.٧٪) patients, and + ٤ positive in ١٠ (١٨.٩٪) (fig. ٢). Thirty-eight (٣٨٪) patients' ascitic fluid had negative dipstick test and PMN count was <٢٥٠/mm^{٣}, but dipstick test was negative in Six(٦٪) patients 'with ascitic fluid PMN cell count was ≥٢٥٠/mm^{٣}.

True positive were ٥٣(٥٣٪), true negative ٣٨(٣٨٪), false positive ٣(٣٪) and false nega-tive were ٦(٦٪) (Table ٢ & Fig. ٣). The sensitivity, specificity, positive predictive value, negative predictive value of leukocyte esterase dipstick test to diagnose SBP were ٨٩.٨٪, ٩٢.٧٪, ٩٤.٦٪, ٨٢٪ respectively (Table ٢ & Fig. ٤).

Results of this study are quite similar to other studies published to date evaluating the use of leukocyte esterase dipstick test in the diagnosis of SBP.

Thévenot T., and his colleague[٣٩] studied Thirty-one unselected consecutive cirrhotic patients with ascites and a total of ١٠٠ paracen-teses were performed. All ascitic fluid was analysed with the reagent strips, leucocyte and polymorphonuclear (PMN) leucocyte cell count. The sensitivity, specificity, positive and

negative predictive values of the strips were ٨٩٪, ١٠٠٪, ١٠٠٪ and ٩٩٪, respectively.

Sarwar S., et al.,[٣٣] studied ٢١٤ paracentesis, SBP was diagnosed in ٣٨ patients whereas ١٧٦ were negative for infection. Strip test was ٩٧.٧٪ sensitive and ٨٩.٤٪ specific with positive predictive value of ٩٠٪, negative predictive value of ٩٧.٧٪ and accuracy of ٩٦.٢٪.

In Do Young Kim[٣٤] study a total of ٧٥ paracenteses in ٥٣ cirrhotic patients with ascites were performed. the sensitivity, specificity, ppv, and npv were ٦٧٪, ١٠٠٪, ١٠٠٪, and ٨٩٪, respectively.

In a study done by Rungsun Rerknimitr et al.,[٣٥]. There were ٢٠٠ consecutive samples from cirrhotic patients who underwent abdominal paracentesis. Urine dipstick was used.

A cell count with differential study was done in all samples. The sensitivity, specificity, positive and negative predictive values and accuracy of ١+ and ٢+ cut off scale to diagnose SBP were ٨٨٪, ٨١٪, ٥٥٪, ٩٦٪ and ٨٣٪ respectively, and ٦٣٪, ٩٦٪, ٨٢٪, ٨١٪ and ٨٩٪ respectively.

In a similar study done by Braga LL., et al.,[٣٦] the sensitivity, specificity, positive predictive value and negative predictive value for the strips were respectively ١٠٠٪, ٩٨.٩٪, ٩٢.٣٪ and ١٠٠٪.

Serkan Torun[٣٧] and his colleague studied a total of ٦٣ consecutive patients with cirrhotic ascites. The sensitivity, specificity, positive and negative predictive values of the leukocyte esterase reagent strips were; ٩٣٪, ١٠٠٪, ١٠٠٪, and ٩٨٪ respectively.

Balagopal et al.,[٣٨] studied ١٧٥ patients with liver cirrhosis and ascites had a sensitivity of ٩٢٪ and specificity of ١٠٠٪.

Hanan H. Soliman et al.,[٣٩] studied samples from ١٠٣ cirrhotic patients for Leucocytic esterase test which had sensitivity, specificity, positive predictive value and negative predictive value of ٩٣.٦٪, ٩٨.٧٪, ٩٥.٧٪ and ٩٨.٧٪ respectively.

Rajput et al.,[٤٠] studied two hundred six cases of cirrhosis and the results of ascitic fluid analysis was sensitivity, specificity, positive predi-ctive value, negative predictive value & accuracy as; ٨٨.٩٪, ٩٨.٧٪, ٧٦.١٪, ٩٦.٢٪ & ٩١.٣٪ respectively.

Nanik Ram Khatwani et al.,[٤٣] studied ninety four patients. SBP was diagnosed in ٥٢ (٥٥.٣٪) patients, ٤٢(٤٤.٧٪) patients were negative for SBP by manual cell count. The sensitivity, specificity, ppv, npv of leukocyte esterase dipstick test to diagnose SBP were ٩٢٪, ٩٥٪, ٩٦٪, ٩٠٪ respectively.

Conclusion

The leukocyte esterase dipstick test has a high sensitivity, specificity, positive predictive value and negative predictive value for diagnosis of spontaneous bacterial peritonitis (SBP) in cirrhotic patients with ascites. It can be used as an easy, rapid, inexpensive bedside test for diagnosis of SBP. A positive test result could be used as an indication for antibiotic therapy and a negative test result could exclude SBP.

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