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# Diagnostic Value of Ascitic Fluid Lactoferrin, Calprotectin, and Calprotectin to Albumin Ratio in Spontaneous Bacterial Peritonitis

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## ABSTRACT

Spontaneous bacterial peritonitis (SBP) is common and dangerous infection that occurs in 8-27% of cirrhotic inpatients with ascites. In the current study we have demonstrated and compared the diagnostic performance of ascitic fluid lactoferrin and calprotectin in the screening and diagnosis of SBP. In addition we have evaluated ascitic fluid calprotectin to albumin ratio as a novel marker in the diagnosis and Prognosis of SBP. In this cross sectional analytic study, ascitic fluids were collected from 87 cirrhotic patients (49 with SBP and 38 without SBP. Calprotectin and lactoferrin were measured in the ascitic fluids by quantitative sandwich enzyme-linked immunosorbent assay (ELISA). Ascitic fluid calprotectin and lactoferrin were increased significantly in SBP group when compared with non SBP group. Moreover, they were correlated in a positive manner with white blood cells (WBCs) count in ascitic fluid, Polymorphnuclear cells (PMN) count, and serum C-reactive protein (CRP). Also, calprotectin to albumin ratio was increased in SBP cases versus non SBP cases. Moreover, calprotectin to albumin ratio was significantly higher among SBP patients who died inside the hospital than patients with SBP who survived. Both ascitic fluid lactoferrin and calprotectin can be used as valuable markers for diagnosis of SBP. However, ascitic fluid calprotectin is more sensitive and more specific than lactoferrin for diagnosis of SBP. Moreover, calprotectin to albumin ratio in ascites is useful in the diagnosis for SBP as well as it provides prognostic information about the in hospital mortality.

## Introduction

Spontaneous bacterial peritonitis (SBP) is a common and dangerous infection that occurs in 8-27% of admitted patients with cirrhosis and ascites (Thuluvath *et al.,* 2001; Garcia-Tsao, 2005). The prevalence of SBP among outpatients cirrhotics is up to 3.5% (Evans *et al.,* 2003).

In a lot of cases, SBP can be asymptomatic and the diagnosis depends on paracentesis

only. The diagnosis of SBP is based on the presence of > 250 polymorphonuclear (PMN) cells/mm$^3$ in the ascitic fluid and no evidence of any intra-abdominal or surgically treatable source of infection (Rimola *et al.,* 2000).

After transporting the ascitic fluid to the laboratory, the number of PMNs in this fluid is measured. False-negative results may occur due to lysis of the PMNs during transport to the laboratory. Manual counting of the PMN in the ascitic fluid is operator-dependent and can delay the diagnosis (Runyon, 2003). An early start of antibiotic therapy is important for the successful treatment of SBP and has been shown to reduce mortality and improve survival (Hoefs *et al.,* 1982). Therefore, the detection and evaluation of new biomarkers can support the rapid diagnosis and management of SBP (Parsi *et al.,* 2008).

Lactoferrin is a protein binder to iron that is found in PMNs and is released on degranulation (Lyer and Lonnerdal, 1993). Previous studies showed that lactoferrin in stool provide a reliable marker of inflammatory diarrhea (Langhorst *et al.,* 2008). The calculation of lactoferrin in ascitic fluid would be considered as biomarker for the presence of PMNs and detection of SBP in cirrhotic patients (Rimola *et al.,* 2000).

Calprotectin, which is protein binder to zinc and calcium, is detected in neutrophils (Schäfer and Heinzmann, 1996), and its amount in the fluids is proportionate to neutrophils entry in the fluid (Jung *et al.,* 2010). Presence of calprotectin in ascites indicates PMN count >250/mm$^3$, and this useful in SBP diagnosis (Burri *et al.,* 2013).

C-reactive protein (CRP) is produced by the liver as a result of activation by interleukin-6 (IL-6). It is an acute-phase protein. The hepatic synthesis of CRP starts 6 to 8 hours after onset of infection (Pfafflin and Schleicher, 2009) and it has been shown that high CRP was associated with an increased risk of infection (Lopes-Ferreira *et al.,* 2003).

In the current study we have demonstrated and compared the diagnostic performance of ascitic fluid lactoferrin and calprotectin in the screening and diagnosis of SBP. In addition we have evaluated ascitic fluid calprotectin to albumin ratio as a novel marker in the diagnostic and prognostic of SBP.

## Materials and Methods

### Patients

In this cross sectional analytic study, 87 cirrhotic patients with ascites (HCV related) were consecutively admitted into Al Rajhy Tertiary Liver Hospital, Assiut University and the Department of Tropical Medicine, Sohag University Hospital, Egypt from April 2015 to September 2015. Patients who had evidence of active infection other than ascitic fluid infection or were pre hospitalized on antibiotic therapy/ SBP prophylaxis or had abdominal surgery within the last 3 months were excluded from the study.

The following evaluations were done: complete history taking; physical examination; laboratory assessment as complete blood count, liver function, serum creatinine, CRP; and abdominal ultrasound. Diagnostic paracentesis were performed under aseptic conditions. Ascitic fluid analysis including physical examination; chemical analysis for protein, albumin, lactoferrin and calprotectin level; cellular examination for total WBCs, PMN count; and bacteriologic examination and culture.

SBP diagnosis depends on the presence of ≥ 250 PMN cells/m$^3$ in the ascites in the absence of secondary peritonitis, irrespective of ascitic fluid culture results (Rimola *et al.,* 2000).

Patients were divided into 49 cases with PMN count ≥250 cells/mm³ in ascitic fluid with or without ascitic fluid positive culture for bacteria (SBP group); 38 cases with PMN count <250 cells/mm³ in ascitic fluid and negative culture (non SBP). SBP group was further subdivided into SBP (culture negative) and SBP (culture positive).

**Sampling and detailed methods**

**Sampling**

Seven ml blood was withdrawn by venipuncture and distributed into three tubes; two ml in Ethylenediaminetetra acetic acid (EDTA) tube for complete blood count (CBC), two ml for prothrombin time and concentration, and three ml delivered into plastic tube and allowed to clot for serum separation. Centrifugation was used to separate non-hemolyzed sera to determine creatinine, CRP, and liver functions (Alanine aminotransferase (ALT); Aspartate aminotransferase (AST); total bilirubin and albumin).

Paracentesis performed under complete aseptic condition to obtain ascitic fluid sample. Ascitic fluid sample was divided into three parts; one part was used for bacteriological culture; second part was used for routine analysis of ascitic fluid, and the third part was aliquoted into two tubes and stored at -80$^0$ C for measurement of lactoferrin and calprotectin.

**Materials and Methods**

Liver function and serum creatinine concentrations were measured on ABX Pentra 400 chemistry autoanalyzer (Horiba Medical, Irvine, California, USA).

Complete blood picture was measured using ABX Pentra XL 80 five differential automated cell counter (Horiba Medical, Irvine, California, USA). Serum CRP was determined using QuikRead go instrument (Orion Diagnostica, Espoo, Finland).

Serum Calprotectin was measured in ascitic fluid by the use of commercially available quantitative sandwich enzyme-linked immunosorbent assays using BioVendor Laboratoni medicina, Czech Republic, Catalog number (Cat No): RD 191217100R).

Serum Lactoferrin was measured in ascitic fluid by the use of commercially available quantitative sandwich enzyme-linked immunosorbent assays using BioVendor Laboratoni medicina, Czech Republic, Catalog number (Cat No): RD 194334200R).

The bacteriologic examination for ascitic fluid was performed using the modified culture method (Bourbeau *et al.,* 1998; Akcam *et al.,* 2006). Briefly, inoculation of 10 mL of the ascitic fluid into blood culture bottles was done. The bottles were kept under both anaerobic and aerobic conditions at 37 $^0$C for about 5 days in BACTEC 9050 automatic blood culture system (Becton-Dickinson Diagnostic Systems, MD, USA).

Each bottle was tested every 10 min by the BACTEC instrument. Positive bottles for bacteria were removed from the BACTEC system. Gram staining and sub-cultured were done to identify the microorganism (Ferrer *et al.,* 1999).

**Statistical analysis**

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS, version 20; SPSS Inc., Chicago, IL, USA) software. Means ± standard deviation/ Error or frequencies were used to show the results. Chi-square tests were used to compare the Proportions. The data were nonparametric.

Mann-Whitney U test was used to compare between the 2 groups in the continuous variables. The correlations between calprotectin or lactoferrin and WBCS, PMN count, and CRP were tested using Spearman's correlation analysis. p values <0.05 were significant. The ability of calprotectin or lactoferrin to detect SBP in patients with liver cirrhosis was tested by receiver operator characteristic (ROC) curve. Graphs were performed using Microsoft Excel.

**Ethics**

The Ethical Committee of the Faculty of Medicine approved this study and all patients provided their written informed consent before enrollment in the study. The study was done according to Helsinki Declaration Guidelines.

**Results and Discussion**

**Demographic data, clinical characteristics, laboratory data, and liver disease severity**

According to ascitic fluid analysis, 49 patients were defined as SBP cases (27 male, 22 females, mean age: $52.78 \pm 4.18$) and 38 patients were defined as non-SBP cases (22 male, 16 females, mean age: $51.74 \pm 6.16$). No significant difference were observed between SBP cases and cases without SBP as regard mean age and gender.

The most common clinical presentation in SBP group was abdominal pain (89.8.7%), followed by fever (65.3%), hepatic encephalopathy (63.2%), abdominal tenderness (55.1%). SBP cases had higher percentage of abdominal pain, fever, and tenderness than non-SBP cases.

Also, presence of internal echoes in ascites in ultra sound and Child Pugh grade C was significantly higher in SBP (p <0.001, p = 0.002) respectively. However, no difference

between the two groups as regard to percentage of hepatic encephalopathy, renal impairment, refractory ascites or the mortality rate (p > 0.05 for each) (Table 1).

The laboratory investigations of the study groups revealed a significant difference between both groups regarding WBCs count, total serum bilirubin, INR, and CRP where these variable were elevated in SBP group (p = 0.014, p = 0.037, p = 0.014, p = 0.001) respectively. No difference were found in other laboratory data between both groups (p >0.05) (Table 2).

Table 3 showed ascitic fluid chemical analysis. There was a difference between the two groups as regards to total leucocytic count and PMN cell count; both were higher in SBP group (p < 0.001 for each).

There was an increase in ascitic fluid calprotectin (ng/ml) in the group with SBP versus non SBP group ($3403.1 \pm 1840.7$ versus $417.8 \pm 185.7$) (p < 0.001). Moreover, significant difference was found between cases with SBP and cases without SBP as regard calprotectin to albumin ratio (p < 0.001). Similarly, ascitic fluid lactoferrin (ng/ml) was significantly increased in SBP group when compared with non SBP group ($178.9 \pm 51.6$ versus $98.4 \pm 37.5$) (p < 0.001).

As regard ascitic fluid culture, only 15 cases out of 49 with ascitic fluid polymorph equal to or more than 250 (30.6%) were positive (classical SBP) and thirty four (69.4%) were negative.

The isolated organisms in SBP group in the current study were *E. coli* in 40%, *Streptococcus pneumonia* in 20%, *Streptococcus thoraltensis* in 20%, and *Staphylococcus aureus* in 20%. All 38 cases with ascitic fluid polymorph less than 250 were culture negative.

## ROC curve analysis of ascitic fluid calprotectin

Using ROC curve, the optimum calprotectin level cut -off points for SBP among patients with liver cirrhosis was 710 (ng/ ml) with a sensitivity and specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy of 95.9 %, 97.4%, 97.9 %, 94.9 %; 96.6% respectively (area under curve: 0.976) (Figure 1).

Ascitic fluid calprotectin in culture positive cases (classical SBP) was (1827.1±1610.6) while in culture negative cases was (3865.7 ± 1758.6) (p value = 0.365).

## Ascitic fluid calprotectin correlations

Ascitic fluid calprotectin was significantly positively correlated with both of ascitic fluid WBC and PMN count ($r = 0.777$; $p < 0.001$) and ($r = 0.784$; $p < 0.001$) respectively. In addition, ascitic fluid calprotectin was positively correlated with serum CRP ($r = 0.448$; $p < 0.001$) (Figure 2).

## Ascitic fluid Calprotectin and liver disease severity

The mean value of calprotectin was significantly higher in Child Pugh grade C (n= 77) when compared with grade B (n =10) (2266.8± 232.1 versus 807.5±479.3) (p value = 0.005). In addition the mean value of calprotectin significantly increased in relation to MELD (2824.0± 378.9; 2534.1± 487.4) for MELD from 19-24; ≥25 versus (245.0± 111.8; 1294.4±1491.6) for MELD ≤ 10; 11-18 respectively (p value = 0.005) (Table 4).

## ROC curve analysis of ascitic fluid calprotectin to albumin ratio

ROC curve analysis suggested that the optimum calprotectin to albumin ratio cut-off points for SBP in cirrhotic patients was 193.3 with a sensitivity, specificity, PPV, NPV and overall accuracy of 93.9%, 100%, 100%, 92.7%; 96.6% respectively (area under curve: 0.966) (Figure 3).

## ROC curve analysis of ascitic fluid lactoferrin

Using ROC curve, the optimum lactoferrin level cut -off points for SBP among patients with liver cirrhosis was 118.2 (ng/ ml) with a sensitivity, specificity, PPV, NPV and overall accuracy of 91.5%, 86.1 %, 89.6%, 88.6%; 89.2% respectively (area under curve: 0.910) (Figure 4).

## Ascitic fluid lactoferrin correlations

Ascitic fluid lactoferrin was positively correlated with both of ascitic fluid WBC and PMN count ($r = 0.621$; $p < 0.001$) and ($r = 0.617$; $p < 0.001$) respectively. In addition, ascitic fluid lactoferrin was positively correlated with serum CRP ($r = 0.302$; $p = 0.024$).

## Ascitic fluid calprotectin to albumin ratio as a predictor of in hospital mortality

Calprotectin to albumin ratio was significantly higher among SBP patients who died inside the hospital compared to that in patients with SBP who have been survived (p value = 0.003), however this difference was not found among non SBP patients (p value = 0.880) (Table 5).

SBP is considered as an essential cause of morbidity and mortality in decompensated cirrhosis. The mortality rate approaches 30 % (Thuluvath *et al.,* 2001). The most common clinical presentation in this study was abdominal pain (89.8.7%), followed by fever (65.3%), hepatic encephalopathy (63.2%), abdominal tenderness (55.1%).

**Fig.1** Receiver operating characteristic curves of ascitic fluid calprotectin in detecting SBP in cirrhotic patients
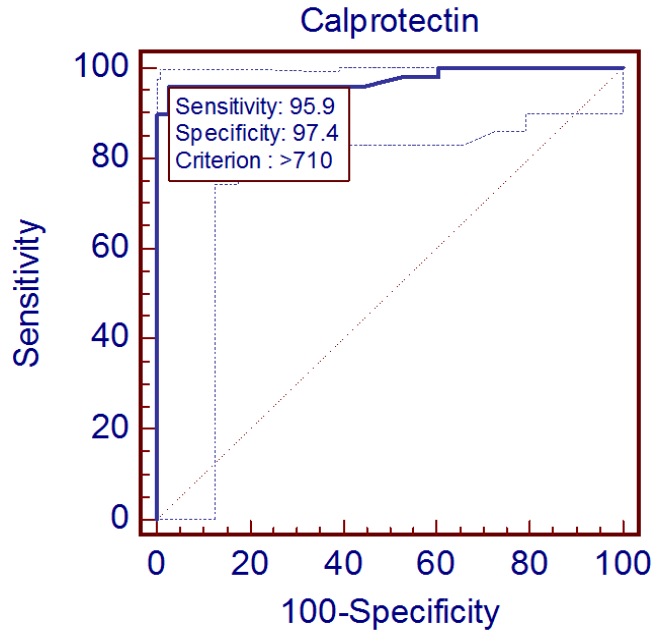


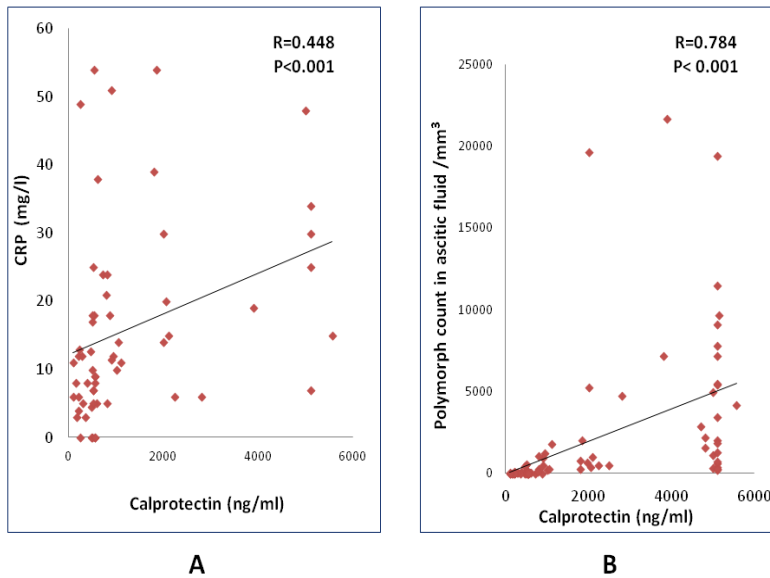**Fig.2** Ascitic fluid calprotectin correlation with serum CRP (A) and ascitic fluid polymorph count (B)



A

B

**Fig.3** Receiver operating characteristic curves of ascitic fluid calprotectin to albumin ratio in detecting SBP in cirrhotic patients
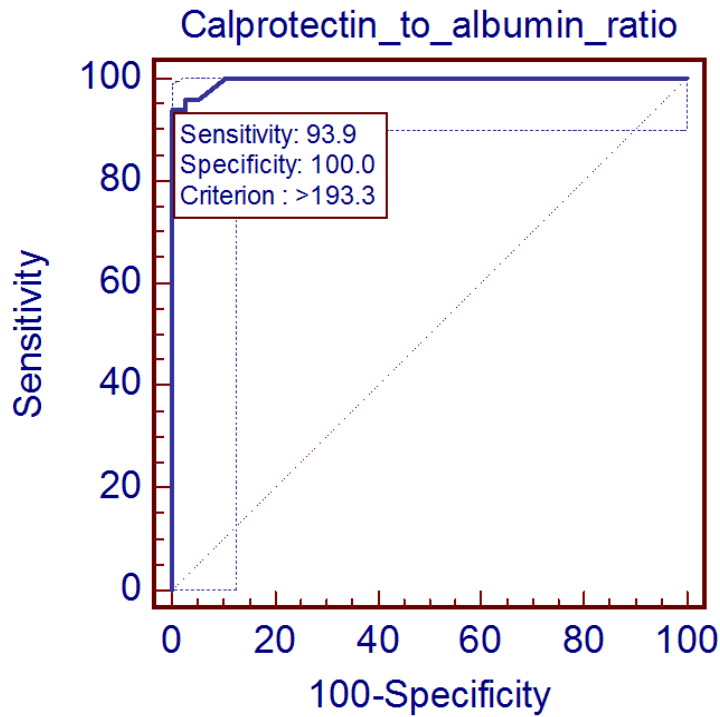


**Fig.4** Receiver operating characteristic curves of ascitic fluid lactoferrin in detecting SBP in cirrhotic patients
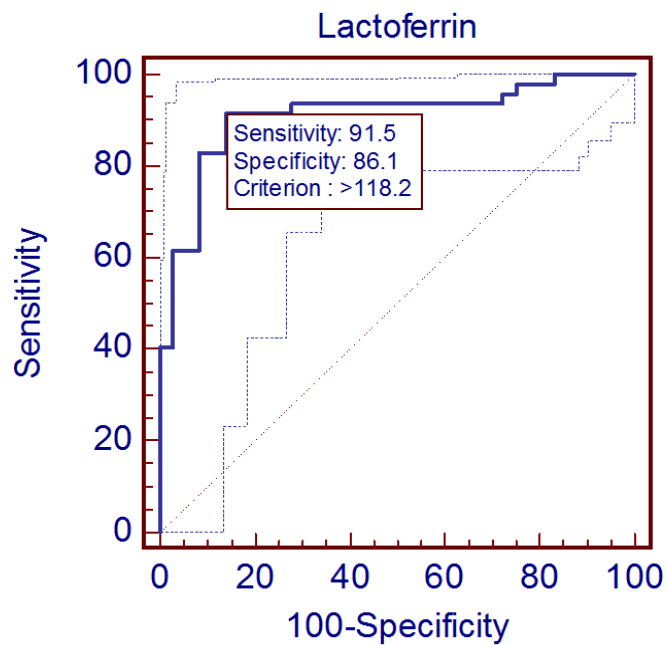
| Table.1 Demographic characteristics, clinical presentations and disease severity of the study groups | | | |
|---|---|---|---|
| **Variable** | **SBP Group (N= 49)** | **non SBP group (N= 38)** | **p value** |
| **Age (yr) ¶** | 52.78± 4.18 | 51.74± 6.16 | 0.352 |
| **Gender:** **Male** **Female** | 27(55.1%) 22(44.9%) | 22(57.9%) 16(42.1%) | 0.794 |
| **Abdominal pain** | 44(89.8%) | 15(39.5) | **<0.001\*** |
| **Fever** | 32(65.3%) | 12(31.6%) | **0.002\*** |
| **Abdominal tenderness** | 27(55.1%) | 9(23.7) | **0.004\*** |
| **Renal impairment** | 15(30.6%) | 10(26.3%) | 0.606 |
| **Refractory ascites** | 22(44.9%) | 18(47.4%) | 0.819 |
| **Internal echos in US** | 15(30.6%) | 1 (2.6%) | **<0.001\*** |
| **Child Pugh grade** **A** **B** **C** | 0(0%) 1 (2.0%) 48(98.0%) | 0(0%) 9(23.7%) 29(76.3%) | **0.002\*** |
| **MELD** **6-10** **11-18** **19-24** **≥ 25** | 0 (0%) 14 (28.6%) 22 (24.9%) 13 (26.5%) | 3 (7.9%) 18 (47.4%) 8 (21.1%) 9 (23.7%) | **0.023\*** |
| **Hepatic encephalopathy** | 31(63.2%) | 21(55.6%) | 0.121 |
| **Mortality rate** | 7(14.3%) | 5(13.2%) | 0.885 |
| **\* Significant, Data are expressed as number and percentage except ¶ Data are expressed as Mean ± SD(Standard Deviation); US: Ultrasound; MELD: Model for End Stage Liver Disease; SBP: Spontaneous Bacterial Peritonitis** | | | |

| Table.2 Laboratory data of the study groups | | | |
|---|---|---|---|
| **Variable** | **SBP Group N= 49** | **Non SBP group N= 38** | **p value** |
| **WBCS (x1000/mm³)** | 9.0±3.9 | 7.0±3.4 | **0.014\*** |
| **RBCS** | 3.3±0.7 | 3.3±0.5 | 0.884 |
| **Hb (g/dl)** | 9.9±1.7 | 10.4±1.8 | 0.219 |
| **PLT (x1000/mm³)** | 99.0±54.3 | 99.8±43.4 | 0.561 |
| **Urea (mmol/ L)** | 14.7±20. 5 | 10.8 ±6.8 | 0.850 |
| **Baseline creatinine (umol/L)** | 140.9±88.7 | 123.5± 56.5 | 0.472 |
| **Na (mEq/L)** | 132.0±7.2 | 132.6±5.8 | 0.477 |
| **K (mEq/L)** | 3.9± 0.8 | 4.3±0.8 | **0.037\*** |
| **Total bilirubin (umol/L)** | 77.7±42.1 | 72.4±81.5 | **0.037\*** |
| **Total protein g/L** | 60.5±9.9 | 65.6±19.3 | 0.237 |
| **Albumin g/L** | 20.8±4.3 | 19.9±3.9 | 0.303 |
| **AST (U/L)** | 72.8±49.8 | 99.7±158.2 | 0.397 |
| **ALT (U/L)** | 53.9±73.2 | 71.0±128.0 | 0.279 |
| **INR** | 1.8± 0.4 | 1.6 ± 0.3 | **0.014\*** |
| **CRP (mg/L)** | 21.2±14.1 | 11. 7±12.9 | **0.001\*** |
| **Hb: Haemoglobin; WBC: White blood cell count; PLT: Platelet count; CRP: C–reactive protein; SBP: Spontaneous Bacterial Peritonitis. Data are expressed as Mean ± SD (Standard Deviation; Na: Sodium; K: Potassium; INR: International Normalized Ratio; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase. \*Significant** | | | |

| Table.3 Ascitic fluid chemical analysis of the study groups | | | |
|---|---|---|---|
| **Variable** | **SBP Group N= 49** | **Non SBP group N= 38** | **p value** |
| **WBCS in ascitic fluid /cm$^3$** | 4352±5990 | 258±146 | <0.001* |
| **Polymorphnuclear leucocyte count in ascitic fluid /cm$^3$** | 3609 ±5164 | 48 ±44 | <0.001* |
| **Ascitic fluid protein g/L** | 10.7±5.2 | 13.0 ±10.2 | 0.080 |
| **Ascitic fluid albumin g/L** | 4.9±3.9 | 5.6 ±3.9 | 0.064 |
| **Calprotectin in ascitic fluid (ng/ml)** | 3403.1 ± 1840.7 | 417.8 ± 185.7 | <0.001* |
| **Calprotectin to albumin ratio** | 1065.1±1033.3 | 97.3± 51.1 | <0.001* |
| **Lactoferrin in ascitic fluid (ng/ml)** | 178.9±51.6 | 98.4±37.5 | <0.001* |
| **SBP: Spontaneous Bacterial Peritonitis; WBCs (white blood cell count); Data are expressed as Mean ± SD (Standard Deviation); * Significant** | | | |

| Table.4 Relation between Calprotectin level and liver disease severity | | |
|---|---|---|
| **Liver Disease Severity** | **Calprotectin level (Mean ± SE)** | **p value** |
| **Child Pugh Grading** **B** **C** | 807.5± 479.3 2266.8± 232.1 | 0.005* |
| **MELD Score** **≤ 10** **11-18** **19-24** **≥ 25** | 245.0± 111.8 1294.4±263.7 2824.0± 378.9 2534.1±487.4 | 0.023* |
| **\* Significant, Data are expressed as Mean ± Standard Error (SE); MELD: Model for End Stage Liver Disease.** | | |

| Table.5 Calprotectin to albumin ratio as a predictor of in hospital mortality | | | |
|---|---|---|---|
| **Variable** | **Mortality (n = 12)** | **Survival (n = 75)** | **p value** |
| **Fate among total cases (n = 87)** | 12 (13.8%) | 75 (86.2%) | 0.568 |
| **Fate among SBP (n= 49)** | 7 (14.3%) | 42(85.7%) | |
| **Fate among non SBP group (n= 38)** | 5(13.2%) | 33(86.8%) | |
| **Calprotectin to albumin ratio among SBP group (n= 49) ¶** | 2244.8± 1453.6 | 868.5± 814.7 | **0.003*** |
| **Calprotectin to albumin ratio among non SBP group (n= 38) ¶** | 101.2±41.4 | 96.7±52.9 | **0.880** |
| ** \* Significant, Data are expressed as number and percentage except ¶ Data are expressed as Mean ± SD (Standard Deviation).** | | | |

These results were approximately near the results of the study conducted by (Runyon *et al.,* 1985) in which fever was the most common feature (67%), followed by abdominal pain (60%), abdominal tenderness (42%) and encephalopathy (57%). In the minority of SBP patients, bacterial culture could yield positive results and the results were delayed for several days (Runyon, 2003). In the present study, 30.6 % of SBP

patients had positive bacterial culture and this result was consistent with the results of (Khalifa *et al.,* 2013) and (Lutz *et al.,* 2015). The isolated organisms in SBP group in the current study were E. coli in 40%. This was in agreement with the results of (Liovet *et al.,* 1997) who stated that single gram negative organism specially E. coli cause most episodes of SBP.

SBP diagnosis depends on ascitic fluid PMN count but this test sometimes lack sensitivity and may delay the diagnosis (Riggo and Angeloni, 2009). The delay in starting antibiotic treatment carries a high mortality rate. So, considerable efforts should be made to search for another test for rapid SBP diagnosis. Several alternative methods including, CRP, WBCs levels measurement, and mean platelet volume have been studied and may play a promising role in supporting the diagnosis of SBP (Abdel-Razik *et al.,* 2014).

Lactoferrin is a protein binder to iron contained in PMNs and is released on degranulation (Parsi *et al.,* 2008). Martin *et al.,* (1995) reported that, lactoferrin levels correlate with absolute neutrophil count in blood samples, and with the presence of neutrocytic inflammation in body fluid. Lactoferrin also has been found to be stable, resistant to degradation when kept for a long time at room temperature. This criterion renders lactoferrin attractive for use in clinical practice (Kayazawa *et al.,* 2002).

In this study, ascitic fluid lactoferrin was assessed in patients with liver cirrhosis in the presence or absence of SBP to evaluate its role in SBP diagnosis. The mean ascitic fluid lactoferrin level was raised in SBP patients versus non SBP group (178.9±51.6 versus 98.4±37.5). Results of the current study confirmed the previous results reported by (Parsi *et al.,* 2008). The elevation of

lactoferrin level in patients with SBP could be explained by the fact that lactoferrin is a major component of specific granules of human PMN leukocytes to be actively secreted by these cells into the environment in response to inflammation, bacterial infection and cytokine stimulation (Birgens, 1985). Ascitic fluid lactoferrin was significantly positively correlated with ascitic PMNs count and the results of our study confirm a previous results reported by (Khalifa *et al.,* 2013).

In the present study by using ROC curve, the optimum lactoferrin level cut-off points for SBP in patients with liver cirrhosis was 118.2 (ng/ ml) with a sensitivity, specificity, PPV, NPV, and overall accuracy of 91.5%, 86.1 %, 89.6%, 88.6%; 89.2% respectively. Parsi *et al.,* (2008) demonstrated 95.5% sensitivity and 97% specificity at a cut-off 242 ng/ml in diagnosis of SBP. Khalifa *et al.,* (2013) reported ascitic lactoferrin concentration ≥270 ng/ml had 96% sensitivity and 95% specificity, 97.96% PPV, 90.5% PPV, and 95.7% accuracy in diagnosis of SBP. However, our results were much lower than the results reported by the previous studies. This may be due to the difference in the level of cut-off points.

In this study, ascitic fluid calprotectin (ng/ml) is significantly increased in SBP group when compared with non SBP group (3403.1 ± 1840.7 versus 417.8 ± 185.7; p <0.001). These results are in consistent with those demonstrated in the study of (Abdel-Razik *et al.,* 2015). In the current study, ascitic fluid calprotectin is correlated in a positive manner with ascitic PMNs count (r =0.784; p <0.001) and these results confirmed the previous results stated by (Burri *et al.,* 2013), and (Rizk *et al.,* 2014).

In the present study using ROC curve, the optimum calprotectin level cut-off points for

SBP was 710 (ng/ ml) with a sensitivity, specificity, PPV, NPV, and overall accuracy of 95.9 %, 97.4%, 97.9 %, 94.9 %; 96.6% respectively (area under curve: 0.976).

Rizk *et al.,* (2014) reported that at a cut-off level of 270 mg/dl, ascitic fluid calprotectin demonstrated 86% specificity and sensitivity of 97.5% for diagnosing SBP. Burri *et al.,* (2013) stated that ascitic calprotectin cut-off level of 0.63 µg/ml gave 95% sensitivity and 89.2% specificity. Abdel-Razik *et al.,* (2015) reported that cut-off value for calprotectin in ascitic fluid of 445 ng/mL had 95.4% and 85.2% for specificity and sensitivity respectively for diagnosing SBP. In the current study, there is a statistically significant difference between cases with SBP and cases without SBP as regard calprotectin to albumin ratio (p value < 0.001). Using ROC curve, the optimum calprotectin to albumin ratio cut-off points for SBP was 193.3 with a sensitivity, specificity, PPV, NPV, and overall accuracy of 93.9%, 100%, 100%, 92.7%; 96.6% respectively (area under curve: 0.966). Calprotectin to albumin ratio was significantly higher among SBP cases who died inside the hospital than in patients with SBP who have been survived (p value = 0.003), however this difference was not found among non SBP patients

Our findings suggest that the ratio of calprotectin to albumin in ascites could be a useful diagnostic test for SBP and provide prognostic information.

Moreover, in the current study, we investigated the role of several other parameters in supporting the diagnosis of SBP. An increase in blood WBC count was seen in SBP when compared to non-SBP group (9.0±3.9 vs. 7.0±3.4), which was also in agreement with the result of (Rizk *et al.,* 2014). In the present study, ascitic fluid analysis at admission revealed that mean total

leucocytic count, PMN and protein were 4352± 5990/mm$^3$, 3609± 5164/mm$^3$ and 10.7± 5.2 gm/dl respectively and these results were much higher than the results of previous study (Rizk *et al.,* 2014). Preto-Zamperlini *et al.,* (2014) stated that patients with SBP demonstrated an elevation in CRP serum levels and he concluded that CRP is an independent predictor in the development of SBP. In this study, we have similar results where serum CRP was significantly increased in the SBP cases versus the non-SBP cases (21.2 ±14.1 vs. 11.7±12.9).

The current study was the first which showed relation between ascitic fluid calprotectin mean level and liver disease severity as assessed by Child Pugh grade and MELD score. The mean value of ascitic fluid calprotectin was significantly higher in Child Pugh grade C when compared with grade B (2266.8± 232.1 versus 807.5±479.3) (p value = 0.005). In addition the mean value of ascitic fluid calprotectin significantly increased in relation to MELD (p value = 0.005). Elbanna *et al.,* (2008) found a positive correlation between ascitic calprotectin and bilurubin (r = 0.81, p < 0.01) and a significant negative correlation with markers of synthetic liver function (albumin: r = - 0.49, p < 0.05 and prothrombin activity; r = - 0.61, p < 0.01).

Limitations of the study: There are some drawbacks in the current study. First, small sample size, and so larger study is needed to determine the reliable cut-off values for ascitic lactoferrin and ascitic calprotectin for SBP diagnosis. Second, the lack of follow up for the patients after discharge from the hospital to identify if calprotectin to albumin ratio in ascites could be a useful prognostic test after hospital discharge.

In conclusion, both ascitic fluid calprotectin and lactoferrin were raised in cirrhotic patients with SBP when compared with

cirrhotic patients without SBP. Moreover, they were positively correlated with ascitic fluid WBCs count, PMN cell count, and serum CRP. Ascitic fluid lactoferrin and calprotectin may be used as valuable diagnostic tests for screening and diagnosing SBP in patients with liver cirrhosis. However, ascitic fluid calprotectin is more sensitive and more specific. In addition, ascitic fluid calprotectin mean level was associated with liver disease severity as assessed by Child Pugh grade and MELD score. Our findings suggest that calprotectin to albumin ratio in ascites could be useful diagnostic test for SBP and can provide prognostic information for in-hospital mortality.

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