Assessment of Ascitic Fluid Calprotectin role as a Marker for Diagnosis and follow up treatment of Spontaneous Bacterial Peritonitis

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Abstract: Background: Spontaneous bacterial peritonitis (SBP) is a potentially fatal condition, characterized by infection of ascitic fluid in absence of any intra-abdominal surgically treatable source of infection. It is the most common infectious complication of cirrhotic patients. SBP is a condition that requires a high index of suspicion, rapid and accurate diagnosis in addition to prompt and effective therapy. It is also characterized by a high recurrence rate within one year of the 1st episode. **Objective:** The goal of the present study was to assess the role of ascitic fluid calprotectin in diagnosis of SBP. **Patients and Methods:** For this purpose, 60 patients with decompensated liver disease were selected. These patients divided into: Non SBP Group: include 30 patients with cirrhotic ascites without clinical or laboratory evidence of spontaneous bacterial peritonitis. SBP Group: include 30 patients with cirrhotic ascites and spontaneous bacterial peritonitis. **Results:** There was highly statistically significance increase in number of cases presented with fever, abdominal pain, abdominal tenderness and upper GIT bleeding in SBP group compared to non SBP group. Splenomegaly and ascitic fluid turbidity were obviously appeared in ultrasound examination of SBP group. **Conclusion:** ascitic fluid calprotectin was significantly elevated in SBP patients in comparison with non-SBP patients. In addition, they also correlate well with the PMNLs count and protein levels in ascitic fluid and reliably diagnose SBP.

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1. Introduction

Ascites is a pathological accumulation of fluid in the peritoneal cavity and it is a common complication in patients with liver cirrhosis which affect 60% of these patients within 10 years during the course of their disease [(1)].

Spontaneous bacterial peritonitis (SBP) is a common bacterial infection in patients with ascites, it is an acute bacterial infection of ascitic fluids which is previously sterile, without any intra-abdominal source of infection. The most common organisms which infect the ascitic fluid are those of normal intestinal flora [(2)].

SBP affect 10-30% of ascetic patients and the mortality rate reach to about 30%, so, it a very common and important cause of mortality in patients with liver cirrhosis and ascites [(3)].

The diagnosis of SBP is based upon the polymorphonuclear leukocyte cell count (PMNLs) more than or equal 250/ µL in ascitic fluid [(2)].

Calprotectin is a calcium and zinc-binding protein and it is detected almost in neutrophils and its presence in body secretions is directly proportional to the influx of neutrophils [(4)].

Calprotectin is present mainly in neutrophils, macrophages and very rare to appear in lymphocytes. Calprotectin account for about 60% of cytosolic proteins of neutrophils [(5)].

Ascitic fluid calprotectin may be helpful in detection of neutrophil count >250/µL, which may help in diagnosis of SBP and this will be easily bed side test and has an important role in rapid management [(6)].

Aim of The Work

The aim of this work is to identify a new marker (ascitic calprotectin) for diagnosis of (SBP) and follow up in liver cirrhosis Egyptian patients.

2. Patients and Methods

Site and time of study: This work was carried out in hepatogastroenerolgy unit at Ain Shams University and Ahmed Maher teaching hospital from September 2017 to April 2018.

The type of Study: Cross sectional study.

Subjects: A total number of 60 patients with cirrhotic ascites were included in our study and were divided into two main groups: Non-SBP Group: include 30 patients with cirrhotic ascites without clinical or laboratory evidence of spontaneous bacterial peritonitis. SBP Group: include 30 patients with cirrhotic ascites and spontaneous bacterial peritonitis.

Diagnostic criteria for SBP: The diagnosis of SBP is made when there is (1) a positive ascetic fluid bacterial culture and (2) an elevated ascitic fluid absolute PMNLs count (i.e., 250cells/ μL) and (3) without an evident intra-abdominal, surgically treatable source of infection [7].

Inclusion criteria: Cirrhotic Patients with and without spontaneous bacterial peritonitis not receiving antibiotics in last one week.

Exclusion criteria: Patients treated by antibiotics before hospital admission. Recent abdominal surgery (< 3 months). Abdominal malignancy (HCC, Colorectal carcinoma, Gastric carcinoma, Pancreatic carcinoma, Cholangiocarcinoma). Patients with diabetes mellitus, heart failure, hematological diseases, patients with hypo or hyperthyroidism and inflammatory bowel disease. Patients who had received anticoagulant medications, nonsteroidal anti inflammatory drugs or oral contraceptive pills.

Ethical clearance: Written informed consent was taken from the subjects to participate in the study. Approval for performing the study was obtained from internal medicine and Gastroenterology departments, Ain Shams university hospitals and Ahmed Maher teaching hospital after taking Institutional Review Board (IRB) approval.

Statistical analysis

Statistical analysis of results.

Patients with and without SBP were compared by using chi square test for categorical variables and two tailed t test for continuous variables.

Table (1): Clinical presentation of the studied groups.

Pre		Control group		Patients group		Test value*	P-value	Sig.
		No.	%	No.	%			
ABD Pain	No	26	86.7%	2	6.7%	38.571	0.000	HS
	Yes	4	13.3%	28	93.3%			
Fever	No	27	90.0%	4	13.3%	35.306	0.000	HS
	Yes	3	10.0%	26	86.7%			
Disturbed conscious level	No	25	83.3%	8	26.7%	19.461	0.000	HS
	Yes	5	16.7%	22	73.3%			
Upper GI bleeding	No	26	86.7%	12	40.0%	14.067	0.000	HS
	Yes	4	13.3%	18	60.0%			
Nausea vomiting	No	22	73.3%	18	60.0%	1.200	0.273	NS
	Yes	8	26.7%	12	40.0%			
Diarrhea	No	24	80.0%	17	56.7%	3.774	0.052	NS
	Yes	6	20.0%	13	43.3%			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant *: Chi-square test

Table (2): Clinical examination of studied groups.

Variables	Non SBP Group (N = 30)		SBP Group (N = 30)		X^2	P. Value
	n	%	N	%		
General examination: 1-Jaundice	13	45.45	12	40.9	0.093	0.760
2-Lower limb edema	19	63.64	22	72.73	0.419	0.517
3-Hepatic Encephalopathy	10	31.82	16	54.55	2.316	0.128
4-Pallor	20	68.18	19	63.64	0.101	0.750
5-Palmer erythema	19	63.64	22	72.73	0.419	0.517
6-Fever	3	9.1	22	72.7	18.427	0.000*
7-Flappy tremors	10	31.8	16	54.5	2.316	0.128
Local examination: **1-liver size** **-Palpable** **-Not palpable**	 1 29	 4.55 95.45	 3 27	 9.1 90.91	0.358	0.550
2-spleen **-Palpable** **-Not palpable** **-Surgically removed**	 20 10 0	 68.18 31.82 0.0	 19 11 0	 63.64 36.36 0.0	0.101	0.750
3-ascites: ***Mild to moderate** ***Tense ascites**	 15 15	 50 50	 14 16	 45.45 54.55	0.091	0.763
4-Abdominal tenderness	0	0.0	26	86.36	3.344	0.000*

***Significant difference (p value <0.05).** P > 0.05: Non significant P < 0.05: Significant P < 0.01: Highly significant

Evaluation of Calprotectin Point of Care (COP) for diagnosis of SBP were done by identification of best cutoff value by using ROC curve and we calculate the sensitivity, specificity, positive and negative predictive values and likelihood ratios. Level of significance were considered at P value of 0.05.

SPSS V 20 soft ware will be used for statistical analysis.

3. Result

This table shows that, lower limb edema, hepatic encephalopathy, jaundice, pallor, flappy tremors, and palmer erythema were main clinical finding in both studied groups, but with no statistically significant difference. But there was highly statistically significance difference between SBP group compared to non SBP groups regarding fever and abdominal tenderness in local examination.

Table (3): Etiology of liver cirrhosis.

Variables	No. of patients (60)	%
Chronic hepatitis C	45	75
Chronic hepatitis B	8	13.63
Non Alcoholic Steatohepatitis	3	4.55
Autoimmune hepatitis	3	4.55
Cryptogenic cirrhosis	1	2.27

This table shows that, chronic hepatitis C related cirrhosis was in 75% of patients (45 patients), while chronic hepatitis B related cirrhosis was in 13.63% of patients (8 patients), with 3 non alcoholic related cirrhosis (4.55%), 3 autoimmune- related cirrhosis (4.55%) and one cryptogenic cirrhosis (2.27%).

Table (4): Ultrasound findings of the studied groups**.**

Variables		Non SBP Group (N = 30)	SBP Group (N = 30)	test	P. Value
Liver	Size -Normal -Shrunken	 1 (4.55%) 29(95.44%)	 3 (9.1%) 27(90.91%)	0.358^{x}	0.459
	Texture -Coarse -bright	 30(100.0%) 0 (0.0%)	 30(100.0%) 0 (0.0%)	0.000^{x}	1.000
	Hepatic focal Lesion	0 (0.0%)	0 (0.0%)	0.000^{t}	1.000
PV	Diameter (cm)	1.4±0.2	1.9±2.6	0.899^{t}	0.374
	Patency -patent -thrombosed	 30(100.0%) 0 (0.0%)	 30(100.0%) 0 (0.0%)	0.000^{x}	1.000
Spleen	size	14.6±1.8	16.02±1.5	2.843^{t}	0.007*
Ascites	turbidity	0 (0.0%)	18 (59.1%)	18.452^{x}	0.000*
	Amount: -mild to moderate -Severe	 15 (50.0%) 15 (50.0%)	 14(45.45%) 16(54.55%)	0.091^{x} 0.091^{x}	.763 0.763

t (Student's t-test). x (chi-square test). *Significant difference (p value <0.05). P > 0.05: Non significant
P < 0.05: Significant P < 0.01: Highly significant

This table show that there was highly statistically significant difference between SBP and non SBP groups as regards splenomegaly and ascitic fluid turbidity which present in the majority of SBP groups, while there was no significant difference as regards to liver size, liver texture and echogenicity, hepatic focal lesion, PV diameter, patency and amount of ascites.

Table (5): Isolated organisms in the culture of ascitic fluid in studied groups.

Organism	Non SBP Group		SBP Group		X2	P-value
	No.	%	No.	%		
No growth	30	100.0%	0	0.0%		
Echerechia coli	0	0.0%	16	54.5%		
Streptococcus viridans	0	0.0%	8	27.3%		
Klebsiella pneumoni	0	0.0%	3	9.1%	44.000	0.000*
Staphylococcus aureus	0	0.0%	3	9.1%		

Table (6): The severity of liver disease assessed by Child-Pough classification among the studied groups.

Child Pugh	Non SBP Group		SBP Group		X2	P-value
	No.	%	No.	%		
Child A	0	0.0%	0	0.0%	0.863	0.353
Child B	14	45.5%	10	31.8%		
Child C	16	54.5%	20	68.2%		

P > 0.05: Non significant P < 0.05: Significant P < 0.01: Highly significant

This table show that, the majority of both groups were child c (68.2% in SBP group and 54.5% in non SBP group) but without statistically significant difference regarding child score.

Table (7): The severity of liver disease assessed by MELD score among the studied groups.

Variable	Non SBP Group	SBP Group	t-test	P value
MELD Score	15.0±5.80	18.70±7.42	2.186	**0.034***

*Significant difference (p value <0.05).
P > 0.05: Non significant P < 0.05: Significant P < 0.01: Highly significant

This table show that, the mean values of MELD score is significantly higher in SBP group than in non SBP group.

4. Discussion

Ascites is one of the most common complications of patients with cirrhosis and its development carries a relatively poor prognosis but the overall course depends on the degree of reversibility of the underlying liver disease and the response to therapy [8].

Spontaneous bacterial peritonitis is defined as an infection of a previously sterile ascitic fluid in the absence of any evident intraabdominal surgical source of infection [9].

It is the most frequent bacterial infection in cirrhosis, accounting for 10 to 30% of all reported bacterial infections in hospitalized patients [10].

It is also one of the potential life threatening complications in ascetic cirrhotic patients with a mortality rate ranging between 30 and 50% [11].

In-hospital mortality for the first episode of SBP ranges from 10% to 50%, depending on various risk factors [12].

The clinical picture of SBP is non-specific and variable, mainly depending on stage at which SBP is diagnosed [13].

The absence of clinical manifestations in some patients with SBP makes the dependence on reliable marker is an important target, taking into consideration that SBP is one of the most frequent and important complications found in cirrhotic patients with ascites [14].

In clinical practice the diagnosis of SBP is based on a polymorph nuclear cell count (PMNLs) that must be greater than or equal to 250 cell/µL in ascitic fluid in absence of intra-abdominal cause of infection [2].

However total leukocytic and PMNs counts in ascitic fluid are not always readily available, the acount of 250 cell/µL or more of PMNLs is highly indicative of SBP and is an indication for antibiotic therapy [2].

Calprotectin, a calcium and zinc-binding protein that belong to the S100 protein family. It is detected almost exclusively in neutrophils, and its presence in body fluids is proportional to the influx of neutrophils. Calprotectin is primarily expressed in neutrophils and macrophages, while it is not usually present in lymphocytes. Calprotectin constitutes up to 60% of soluble protein content in the cytosol of neutrophil granulocytes [5].

A high level of calprotectin reportedly exists in extracellular fluid during various inflammatory conditions, such as SBP [4].

Ascitic fluid calprotectin reliably predicts PMNLs count >250 cell/ µL which may prove useful in the diagnosis of SBP, especially with a readily available bedside testing device [6].

Whereas, a delay in antibiotic therapy entails a high mortality rate. Considerable efforts therefore, have been placed in developing a rapid and reliable tests for the diagnosis of SBP.

So this study was conducted to estimate the role of ascitic fluid calprotectin level for diagnosis of SBP and to identify a cut - off level of ascitic fluid calprotectin that can be used for development of a rapid bed side test.

As regarding to residence and occupation among both SBP and non SBP patients, there was no significant statistical difference between them, this is in agreement with ***Desai et al.*** [15] who stated that no difference between patients according to residence, occupation, habit, DM and HTN.

The most common clinical presentation in patients with SBP in our study was fever (72.7%), followed by abdominal pain (59.1%), hepatic encephalopathy (54.5%) and upper GIT bleeding (40.9%). This results were consistent with the study conducted by ***Runyon et al.*** [16] in which fever was the most common features followed by abdominal pain and encephalopathy.

In clinical presentation of studied patients, fever was detected in (72.7%) of SBP group with a highly statistical significant difference as compared to non SBP (p = 0.00), similar results were obtained in a studies by ***Mchutchinson and Runyon*** [17] and ***Wallerstedt et al.*** [18] who reported that fever was detected in 66% of SBP patients, also this goes in agreement with ***Paul et al.*** [19] who detected that most patients of SBP have signs clearly suggestive of peritoneal infection, especially fever, so fever is considered one of the characteristic clinical sign of SBP.

Also, patients with SBP presented by abdominal pain (59.1%) with a highly significant difference (p value 0.00) compared to non SBP group (9.1%) and these results were consistent with ***Wallerstedt et al.*** [18] who stated that abdominal pain was detected in (54.5% and 70%) of SBP cases respectively.

Hepatic encephalopathy was detected in (54.5%) of SBP and (31.8%) of non SBP cases with no statistically significant difference between both groups, these results were resemble to that reported by ***Wallerstedt et al***. [18] ***and Nobre et al.*** [12] who stated that, there was no difference in incidence of hepatic encephalopathy in cases with and without SBP.

In the present study, (60 %) of SBP cases had gastrointestinal bleeding with statistically significant difference between both groups (p = 0.000), this was close to that reported by ***Wallerstedt et al.*** [18] who stated that 55% of SBP cases had gastrointestinal bleeding.

In our study, the most obvious clinical findings were lower limb edema, palmer erythema, pallor, hepatic encephalopathy, jaundice and flappy tremor (68%,68%,65%,43%,43%,43% respectively) were the main clinical finding in both studied groups with no statistically significant difference between SBP and non SBP groups, similar results were obtained in a study by ***Paul et al.*** [19] who state that the lower limb edema, hepatic encephalopathy, jaundice are common clinical presentation in decompensated cirrhotic patients with ascites.

Our study showed that, 86.36% of SBP group had abdominal tenderness while no patients had abdominal tenderness in non SBP group with highly statistically significant difference in between both groups (p =0.000).

This was in line with ***Wallerstedt et al.*** [18] who reported that abdominal tenderness were more common in patients with SBP (p<0.01).

As regards the cause of liver cirrhosis in our study, we found that chronic hepatitis C is the cause in (75% of patients), chronic hepatitis B is the cause in (13.63% of patients) non alcoholic- related cirrhosis (4.55%), autoimmune-related cirrhosis (4.55%) and cryptogenic cirrhosis (2.27%). This results were consistent with ***Yousra et al.*** [20] who conclude that hepatitis C virus (HCV) infection is the most common cause of cirrhosis in Egypt with the highest prevalence rate worldwide.

In the present work, we found that the majority of SBP were child – paugh class C (68.2%) but without significant difference in between SBP and non SBP groups (p = 0.353). This result matched with that reported by ***Cirera et al.*** [21] who reported that about 70% of patients who developed SBP had Child class C.

Also, ***Kraja et al.*** [22]***, Obstein et al.*** [23] observed that individuals with moderate to high MELD score present a substantially greater risk for the development of SBP.

Regarding of the parameters of laboratory investigations in this study, SBP group showed highly statistically significant increase in serum total leucocytic count when compared to non-SBP groups (p = 0.000).

In the present study, there was no significant difference between SBP and non-SBP groups (p = 0.222) as regard hemoglobin level. This agreed with ***Coşkun et al.*** [24] who stated that patients with SBP has normal hemoglobin and does not affected by ascitic fluid infection, but the hemoglobin value may be related to the severity of the liver disease which reported by ***Paul et al.*** [19].

Also ***Obstein et al.*** [23] found that, SBP patients had higher serum bilirubin than patients without SBP. Also ***Tsung et al.*** [25] reported that a higher level bilirubin in SBP patients showed higher recurrence and mortality.

In the current study, serum albumin level in SBP groups was statistically significantly lower than in non -SBP group (p = 0.00). This result goes in agreement with ***Ruiz et al.*** [26]***,*** who found that patients with SBP frequently develop a rapidly progressive impairment in systemic hemodynamics, leading to severe hepatic failure.

Regarding prothrombin time, it was significantly prolonged in SBP patients more than non SBP patients (p value 0.005), this result was consistent with ***Umgelter et al.*** [27]***,*** who reported that, there was a high prevalence of disturbance in prothrombin time among patients suffering from SBP.

Ascitic fluid total protein in our study was statistically significant high in SBP patients than in non SBP patients (p =0.000). This result was consistent with ***Abdel-Razik et al.*** [28] who found that, patients with SBP has an obvious increase in ascitic fluid total protein which has an important role in the inflammatory process in SBP, so it can be measured as an inflammatory marker in the early phase of the illness.

On the other hand this result disagree with ***Paul et al.*** [19] who denoted that patients with poor synthetic function have diminished level of protein in ascitic fluid that correlate with low level of opsonization and this play a role in SBP susceptibility and denoted also ascitic fluid total protein < 1 g/dl is important predictor for SBP.

Also ***Abdel-Razik et al.*** [28] show that serum procalcitonin and ascitic calprotectin were significantly higher in SBP patients than in non-SBP patients.

Further analysis of results reveal highly statistically significant positive correlation between ascetic fluid calprotectin and ascitic fluid TLC and PMNLs among SBP group (p = 0.000). Similar result were obtained by ***Soyfoo et al.*** [5] who concluded that calprotectin is detected almost exclusively in neutrophils, and its presence in body fluids is proportional to the influx of neutrophils. And goes also in agreement with ***Burri et al.*** [6] who say that ascetic fluid calprotectin helpful in detection of neutrophil count.

The present study showed significant positive correlation between ascitic fluid calprotectin and MELD score values among SBP group (p =0.000) this result was consistent with ***Abdel-Razik et al.*** [28] who reported that ascitic calprotectin appears to provide satisfactory diagnostic markers for the diagnosis of SBP and its level in ascitic fluid help in detection of the severity of the liver disease.

The present study demonstrated that, ascitic fluid calprotectin at a cutoff value of 3.5 ng/ml, had 96.67% sensitivity and 96.7% specificity with positive predictive value 96.7 % and negative predictive value 96.6% in diagnosis of SBP.

This prospective study evaluated the diagnostic utility of measuring ascitic fluid calprotectin to identify ascitic PMNLs count >250/ µL in patients referred to paracentesis, we found that Patients with an elevated PMNLs count (>250/ µL) had higher ascitic calprotectin levels than those with normal cell counts; this finding indicates that ascitic calprotectin levels correlate well and reliably with PMN count.

It is clinically significant that calprotectin levels in ascitic patients can identify elevated PMNLs counts using ELISA methods. Indeed, ascitic calprotectin may serve as a good marker for PMNLs count and would be amenable to routine SBP screening, especially when measured by a bedside test.

After receiving intravenous antibiotic according to hospital protocol (claforan 2g/ 8 hours for 5 days) or according to culture results we found that ascitic fluid calprotectin was high in SBP group before treatment and was directly proportional to the level of PMNLS in ascitic fluid and with clinical improvement evidenced by ascitic fluid analysis after 72 hours we found marked drop in the level of both ascitic calprotectin and PMNLS which make it a good marker for diagnosis and follow up treatment in patients with spontaneous bacterial peritonitis.

There are several limitations to the current study: First, we included all patients with ascites, irrespective of the etiology, and it may be that our results cannot be generalized to all patients with liver cirrhosis. Second, our sample size was small and larger studies are needed to evaluate this test in different clinical settings and to establish a reliable cut-off for ascitic calprotectin for optimal identification of PMNLs counts >250/µL.

5. Conclusion

Ascitic fluid calprotectin had high sensitivity and specificity in diagnosis of SBP. Ascitic fluid calprotectin might be valuable for rapid diagnosis of SBP and also for detection of severity of liver cirrhosis.

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