

# Transforming Growth Factor Beta 1 in the Ascitic Fluid as Predictor for Spontaneous Bacterial Peritonitis in Cirrhotic Patients due to Hepatitis C Virus infection in Suez Canal University Hospital

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Key words:  
Ascites, ascitic TGF- $\beta$ ,  
polymorphonuclear  
leukocyte, Spontaneous  
bacterial peritonitis

**Background and study aim:** Spontaneous bacterial peritonitis (SBP) is one of the complications of decompensated liver cirrhosis and ascites. A polymorphonuclear leukocyte (PMNL) count of 250/mm<sup>3</sup> or higher is the gold standard for diagnosing SBP. Accurate and early detection of SBP is critical to reduce mortality and complications in patients with cirrhosis. This study aimed to see how accurate ascitic fluid Transforming growth factor- $\beta$  [TGF- $\beta$  1] is a predictor for the detection of SBP.

**Patients and Methods:** Eighty-two Egyptian patients with liver cirrhosis and ascites were enrolled; these patients were divided into two groups: 42 patients with SBP and 40 patients without SBP based on an elevated ascitic PMNL count of 250

cells/mm<sup>3</sup> or more. Ascitic fluid samples were examined in all patients for PMNL count, culture, chemistry, and Transforming growth factor- $\beta$  [TGF- $\beta$ ] concentrations. The work was carried out in the Internal Medicine Department of Suez Canal University Hospital.

**Results:** TGF- $\beta$ 1 levels in the ascitic fluid were correlated significantly with ascitic fluid PMNLs and significantly higher in patients with SBP than non-SBP ( $P < 0.001$ ), with the best cutoff value for the detection of SBP of 151.5 ng/ml with a sensitivity of 100 % and a specificity of 100%.

**Conclusion:** Elevated ascitic fluid TGF- $\beta$ 1 levels in cirrhotic patients are a diagnostic and reliable marker for the detection of SBP.

## INTRODUCTION

Cirrhosis is considered an immunocompromised state that leads to a variety of infections, accounting for around 30% of all mortality [1]. Infections account for a 4-fold increase in mortality among patients with liver cirrhosis [2]. Spontaneous bacterial peritonitis (SBP) is a leading cause of morbidity and mortality in cirrhotic patients with ascites and it is thought to impact 10–30% of cirrhotic patients admitted to the hospital with ascites leading to a death rate approaching 30% [3]. In hospitalized patients with cirrhosis, bacterial infections account for 32% to 34% of them, and infection affects 45% of those with gastrointestinal bleeding [2]. SBP is more common in

hospitalized patients who have routine paracentesis (12%), while it is diagnosed in up to 3.5 percent of patients on outpatient visits [2,3]. With early diagnosis and treatment, the death rate has declined to around 20% [4]. On the other hand, patients have a bad prognosis after SBP with a 6-month mortality rate of 50% after the first diagnosis of SBP [5]. Because of the poor prognosis and outcome, it is critical to diagnose SBP as soon as possible [6]. In patients with liver cirrhosis, SBP is diagnosed when the polymorphonuclear (PMN) cell count in ascitic fluid reaches 250 cells/mm<sup>3</sup>, ascitic fluid cultures show only a single organism, and other types of peritonitis have been ruled out [7]. For early detection of SBP,

several tests have been created and examined, Calprotectin, Lactoferrin, Hepcidin, lipopolysaccharide-binding protein, and Complement 3 are some of these markers [8]. Unfortunately, their diagnostic accuracies are limited, and their usage depends on laboratory personnel and commercially available reagents/components. Therefore, there is still a clinical need for an accurate and convenient method of rapid diagnosis of SBP [9].

Transforming growth factor-beta (TGF- $\beta$ ) is a group of polypeptide growth factors that have a variety of functions and seems to be a specific inhibitor of hematopoietic cells, according to various studies [10]. It affects the proliferation of peritoneal exudates macrophages and causes apoptosis in human lymphocytes and hepatocytes [11]. TGF- $\beta$  levels have been associated with a variety of illnesses, including atherosclerosis and fibrous diseases of the kidney, liver, and lungs. The persistence of chronic inflammation in liver illnesses, such as chronic viral hepatitis, is a crucial factor in dictating the shift in the TGF-signaling pathway from tumor suppression to fibrogenesis, which accelerates liver fibrosis and raises the risk of HCC [12]. The role of TGF- $\beta$  1 in the progression of liver fibrosis has been widely studied. This cytokine promotes the conversion of hepatic stellate cells to myofibroblast-like cells and the creation and breakdown of extracellular matrix proteins [13]. The present work aimed at assessing the ascitic fluid TGF- $\beta$  1 as a reliable diagnostic test marker for diagnosing spontaneous bacterial peritonitis (SBP).

## PATIENTS AND METHODS

This study was an analytical cross-sectional study, carried out at the inpatient ward of the Internal Medicine Department of Suez Canal university hospital. The study included 82 Egyptian patients with liver cirrhosis and ascites aging 18 - 65 years of Both female & male gender, Patients were divided into 2 groups:

- 1- Group A included 40 Patients with liver cirrhosis and ascites without SBP.
- 2- Group B included 42 Patients with liver cirrhosis and SBP based on the clinical presentation and Ascitic fluid (AF) PMNL of 250/mm<sup>3</sup> or higher and had not yet begun antibiotic treatment for SBP.

The criteria for liver cirrhosis diagnosis in the patients that were included in this study include clinical and laboratory and radiological, clinical criteria include clubbing, palmar erythema, spider nevi (angiomas), gynecomastia, feminizing hair distribution, testicular atrophy, small irregular shrunken liver, anemia, caput medusae, drowsiness and asterixis in encephalopathy, Jaundice, ascites, leukonychia, lower limb edema and bruising. Patients were categorized regarding Child-Pugh classification [by three biochemical variables (serum albumin, bilirubin, and prothrombin time (international normalized ratio, INR) in addition to the presence or absence of ascites and clinical signs of encephalopathy]. Patients were scored as follows: 5 – 6 as class A, 7 – 9 as class B, and 10 – 15 as class C. All patients involved in the study were subjected to a thorough medical history and physical examination, and abdominal ultrasound (liver & presence of hepatic focal lesions, spleen, gallbladder, ascites), and a laboratory test that included a full blood count, liver profile, creatinine, and ascitic fluid analysis (WBCs, protein, bacteriologic culture with sensitivity and TGF-B1 level). To diagnose SBP, the presence of 250 cells/mL PMN in the ascitic fluid, with or without a positive ascitic fluid culture in the absence of hemorrhagic ascites and secondary peritonitis.

**Exclusion criteria** included patients who received antibiotic treatment two weeks before hospital admission or treatment that can modify the level of TGF beta 1 (steroids and non-steroidal anti-inflammatory or immunosuppressive drugs). Also, patients with Hepatocellular carcinoma, systemic infection, diabetes mellitus, neoplastic disorders, heart failure, and hematological disorders were ruled out.

## Sampling

1. Venipuncture was used to obtain 5 milliliters of blood, 1 milliliter was placed in an EDTA tube for CBC, and 4 milliliters were placed in a plastic tube and allowed to clot. Centrifugation was done to separate non-hemolyzed sera for detecting creatinine and liver functions (ALT, AST, total bilirubin, and albumin).
2. Paracentesis to obtain an ascitic fluid sample from a puncture site in the left or right lower quadrant under aseptic conditions while the patient was supine. All diagnostic samples

were obtained at the bedside and processed by laboratory personnel without delay.

### Methodology:

- 1- Blood glucose, liver profile, and creatinine concentrations were tested using commercially available reagents and an enzyme-based kit on a Dimension Xpand plus chemistry analyzer (Roche Diagnostics, Basel, Switzerland).
- 2- TGF beta 1 ELISA Kit was used to assess TGF beta 1 in the ascitic fluid using an enzyme-linked immunosorbent assay.

### Statistical analysis [14][15]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. The significance of the obtained results was judged at the 5% level.

## RESULTS:

### Patient characteristics:

This study includes 82 patients with chronic liver disease and ascites, the final diagnosis (SBP) was made. According to ascitic fluid analysis and clinical data, they were divided into a SBP group including 42 patients (29 males and 13 females) and a non-SBP group of 40 patients (24 males and 16 females). 37 (45.1%) of the patients were classed as stage B, and 45 (54.9%) of the patients

were classified as stage C, according to the Child-Turcotte-Pugh Score (Table 1).

### Laboratory and ascitic fluid analysis:

there was a marked increase in total leukocytic count, bilirubin, creatinine, albumin, ascitic fluid LDH, and ascitic fluid TLC compared to the non-SBP group (10.5 vs. 7.1; 5 vs. 2.6; 2.2 vs. 1.8; 4.0 vs. 2.3; 388.7 vs. 93.9 and 9336.5 vs. 220.9 respectively)

There were no significant differences between hemoglobin (Hb), platelet count, ALT, AST, prothrombin time, and Ascitic fluid Glucose in both groups (10.4 vs. 9.8; 168.4 vs. 125.6; 32.1 vs. 47.7; 62 vs. 97.8; 20.5 vs. 17.1 and 120.6 vs. 135.7 respectively) (Table 2).

### Diagnostic value of ascitic TGF $\beta$ 1:

Analyzing the given results through the Receiver Operating Characteristic (ROC) curve; it is revealed that a cutoff point of 151.5 ng/mL was statistically 100% sensitive and 100% specific in diagnosing SBP for patients in the current study (figure 1).

### Relation between TGF- $\beta$ 1 and stage of liver disease:

TGF- $\beta$ 1 was higher in child class C patients than in class B patients by (194.7) and (149.6) respectively (figure 2)

### Relation between TGF- $\beta$ 1 and SBP:

There was a positive correlation between the level of ascitic fluid TGF- $\beta$ 1 and SBP because TGF- $\beta$ 1 is higher in SBP than in non-SBP by (264.2 $\pm$ 40.4) and (80 $\pm$ 17.2) respectively (Table 3).

**Table (1):** Baseline characteristics of patients with liver cirrhosis

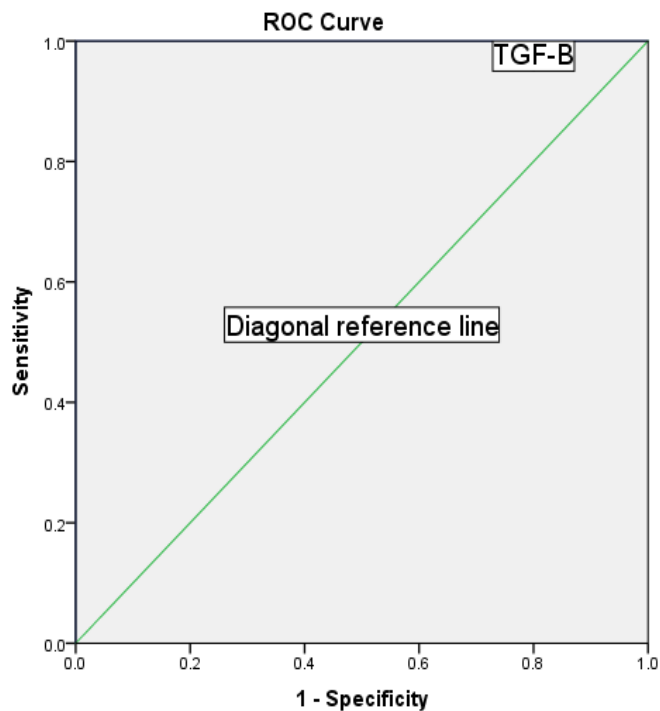
Parameters	No. of patients	Percent
<b>Sex</b>		
Male	53	64.6
Female	29	35.4
<b>Child-Turcotte-Pugh class:</b>		
Child B	37	45.1
Child C	45	54.9

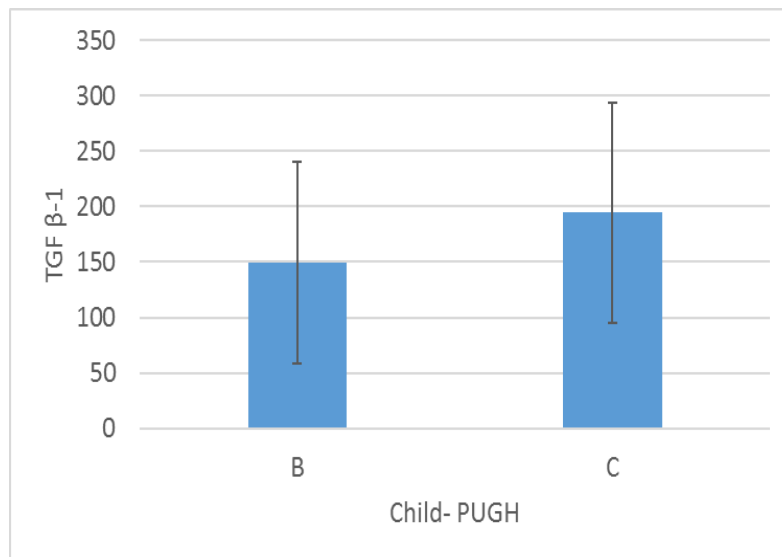
**Table (2):** Biochemical parameters in the studied groups.

	SBP (n=42)		No SBP (n=40)		P value
	Mean	Standard deviation	Mean	Standard deviation	
<b>Hb</b> (gm/dl)	10.4	2.3	9.8	2.1	0.316
<b>TLC</b> ( $10^3$ /cmm)	10.5	7.0	7.1	6.0	0.006*
<b>PLT</b> ( $10^3$ /cmm)	168.4	158.8	125.6	63.4	0.207
<b>ALT</b> (U/L)	32.1	21.0	47.7	102.5	0.791
<b>AST</b> (U/L)	62.0	49.7	97.8	286.5	0.856
<b>Total Bilirubin</b> (mg/dl)	5.0	4.7	2.6	3.3	0.003*
<b>Creatinine</b> (mg/dl)	2.2	0.5	1.8	1.1	0.003*
<b>Albumin</b> (gm/dl)	4.0	0.8	2.3	0.4	<0.001*
<b>Ascitic Glucose</b> (mg/dl)	120.6	80.8	135.7	41.0	0.052
<b>Ascitic LDH</b> (U/L)	388.7	701.3	93.9	52.6	<0.001*
<b>Ascitic TLC</b> (cell/cmm)	9336.5	19728.4	220.9	100.2	<0.001*
<b>P.T.</b> (per second)	20.5	8.6	17.1	4.4	0.062

\* Statistically significant at  $p < 0.05$  (Mann-Whitney test)

**Hb:** hemoglobin, **TLC:** total leukocytic count **ALT:** alanine aminotransaminase, **AST:** aspartate aminotransferase, **LDH:** lactate dehydrogenase

**Figure (1):** Receiver Operating Characteristic Curve for TGF against SBP.



**Figure 2:** The relation between TGF-β1 and child classification

**Table 3:** TGF-β1 Frequency in SBP and non- SBP.

	SBP (no=42)	No SBP (no=40)	P value
TGF-β1 (Mean ±SD)	264.2±40.4	80.0±17.2	<0.001*
	Frequency		Percent
TGF-β1 (Mean ± SD, range)	174.4±97.7		59-350

\* Statistically significant at  $p < 0.05$

## DISCUSSION

Patients with liver cirrhosis often have ascites, which can enhance bacterial translocation and increase the risk of SBP [16]. SBP is uncommon in patients undergoing routine follow-up on outpatient visits, but when it does develop, it frequently necessitates hospitalization to manage the course of the disease [17]. Spontaneous bacterial peritonitis (SBP) is a significant cause of morbidity and mortality in cirrhotic patients with ascites. Pathophysiology of SBP in patients with cirrhosis is regarded to be the main outcome of bacterial translocation (BT). The BT is the condition through which viable or nonviable bacteria and bacterial products (bacterial DNA or endotoxins) traverse across the intestinal lumen and get to the mesenteric lymph nodes or extraintestinal. Bacterial translocation also is participated in augmenting the hyperdynamic state of cirrhosis and in the progression of hemostasis disorders [8].

SBP is diagnosed when the PMN leukocyte cell count in ascitic fluid exceeds 250/L, however, this test is time-consuming and subjective. the diagnosis of SBP still depends on diagnostic paracentesis [8]. It is an invasive maneuver with

some complications. Therefore, there is a need for other noninvasive diagnostic tools.

Ascitic TGF-β1 reliably predicts PMN count  $>250/\mu\text{L}$  which may prove helpful in the diagnosis of SBP.

In this cross-sectional analytical study, which was conducted on 82 patients with liver cirrhosis and ascites, The majority of the patients were in their fourth to sixth decades, which was consistent with the average age ( $53.9 \pm 7.3$  years) reported by Rizk et al. [9]. SBP was more common in males (69%) than in females (31%) which was consistent with Reiberger et al. [18] who also found a 68% male presence of SBP. Our study's male predominance could be related to a higher incidence of bilharziasis and HCV in our area. hemoglobin levels were higher in patients with SBP in comparison with non-SBP patients ( $10.4 \pm 2.3$  vs.  $9.8 \pm 2.1$ ) [table 2] in contrast to Syed et al. who found a mean hemoglobin level of  $9.6 \pm 2.5$  gm/dl in SBP patients and  $9.4 \pm 2.3$  in non-SBP patients [19]. In this study, an increase in TLC count was observed in SBP versus non-SBP (10.5 vs. 7.1) [Table 2], which is consistent with Syed et al. findings [19]. There was no considerable

reduction in platelets in the SBP group compared to the non-SBP group (168.4 vs. 125.6) which contradicts the findings of Ajitpal et al. [20], who observed that platelet count decreased in SBP patients compared to non-SBP patients [table 2].

The SBP group had a significantly higher creatinine concentration than the non-SBP group ( $2.2 \pm 0.5$  vs.  $1.8 \pm 1.1$ ). This is consistent with the findings of Ajitpal et al. [20], who found that serum creatinine levels were substantially higher in patients with SBP than in those without ( $2.44 \pm 0.84$  vs.  $1.8 \pm 1.35$ ,  $p < 0.05$ ).

in a meta-analysis, the negative likelihood ratio for SBP if the PMN cell

count was greater than  $250/\text{mm}^3$  was 0.2 [24]. Another study by El-Gendy et al.,

reported that an ascitic fluid PMN cell count higher than  $200/\text{mm}^3$  had a sensitivity and specificity of 100% in the diagnosis of SBP patients [25]. These data were in agreement with our results as we found that there was a marked rise in TLC and LDH in ascitic fluid ( $9336.519.728$  vs.  $220.9 \pm 100.2$  for TLC and  $388.7701.3$  vs.  $93.952.6$  for LDH) [table 2]. Ascitic fluid analysis at admission by Syed et al. [19] showed mean TLC, polymorphonuclear (PMN), and protein as  $903.34 \pm 3342/\text{mm}^3$ ,  $411.62 \pm 1109/\text{mm}^3$ , and  $1.18 \pm 0.746 \text{ gm/dl}$  respectively. Patients with SBP exhibited considerably higher ascitic fluid LDH ( $p = 0.001$ ), according to Sandhya et al. [21] In the SBP group, the median (IQR) was 201 IU/L (118-921.5), compared to 74 IU/L (48-128) in the non-SBP group. However, our findings were significantly lower than those reported in prior studies. This could be related to the fact that the immunological status and etiology of cirrhosis in individuals in our study (due to HCV infection) differed from those in other studies (alcoholic cirrhosis).

In our study, we found a significant positive correlation between the existence of SBP with elevated ascitic levels of TGF-1 (Table 3) with a significant positive correlation being observed between TGF- $\beta$ 1 and ascitic fluid PMNs (figure 1). Analyzing the given results through the Receiver Operating Characteristic (ROC) curve; it is revealed that a cutoff point of 151.5 ng/mL was statistically 100% sensitive and 100% specific in diagnosing SBP for patients in the current study (figure 1). This is in agreement with Dasarathy who explained this finding by the

fact that TGF-  $\beta$  is one of the inflammatory mediators in patients with liver disease and this may be the cause that it increases in liver disease patients generally and in SBP patients especially [22].

In this study, TGF- $\beta$ 1 levels in the ascitic fluid were observed to be substantially higher in child C patients than in child B patients ( $194.799.6$  vs.  $149.690.7$ ,  $P = 0.037$ ) (figure 2) which may be due to the increased levels of cytokines released from liver cell damage and deteriorated liver cell functions and this is consistent with Claudio et al. who reported that Increased amounts of anti-inflammatory substances in both noninfected cirrhotic patients and SBP patients imply that these molecules regulate the inflammatory process in liver cirrhosis patients [23].

The diagnostic value of detecting TGF- $\beta$ 1 in ascites as a Predictor for Spontaneous Bacterial Peritonitis in Cirrhotic Patients was investigated in this study, and the following new information was discovered: Patients with a high PMN count ( $>250/\text{L}$ ) showed greater ascitic TGF- $\beta$ 1 levels than those with normal cell counts, implying that ascitic TGF- $\beta$ 1 levels correlate well and consistently with PMN count. TGF- $\beta$ 1 levels in ascitic individuals can be used to detect higher PMN counts using ELISA methods, which is clinically significant. Indeed, ascitic TGF- $\beta$ 1 could be used as a surrogate marker for PMN count and could be used in routine SBP screening, particularly if assessed by a bedside test.

The current study has several limitations that should be considered. our sample size was modest, and bigger studies are needed to assess this test in various clinical contexts and to develop a consistent ascitic TGF-  $\beta$ 1 cut-off for optimum detection of PMN counts  $>250/\text{L}$ .

In conclusion, TGF- $\beta$ 1 levels in the ascitic fluid are considerably higher in SBP patients than in non-SBP patients. They also reliably diagnose SBP and correlate well with PMN count and LDH levels in ascitic fluid. It may be beneficial to implement TGF- $\beta$  in future diagnostic algorithms for early diagnosis of SBP in patients with liver cirrhosis.

#### ACKNOWLEDGEMENT

We would like to express our sincere gratitude to the patients and staff of the Internal Medicine Department, and to the laboratory technicians, for their valuable efforts.

**Funding:** None.

**Conflict of interest:** Authors declare no conflict of interest related to this article.

**Ethical aspect:**

The study was approved by the institutional ethical committee at the Faculty of Medicine, Suez Canal University, the aim, and benefits of the study were explained individually to each participant and after approval, informed written consent was obtained from each participant.

**Research Highlights:**

1. Elevated ascitic fluid TGF- $\beta$ 1 levels in cirrhotic patients are a diagnostic for SBP.
2. TGF- $\beta$ 1 is a surrogate marker for PMNL.
3. This study highlights the potential role of TGF- $\beta$ 1 in inflammatory process.
4. TGF- $\beta$ 1 is a promising laboratory test for SBP.

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