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Diagnostic parameters for spontaneous bacterial peritonitis in Egyptian patients with and without underlying malignancies

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Abstract

Background: Ascites can present a challenging diagnostic problem. Bacteria causing spontaneous bacterial peritonitis (SBP) may differ in patients with and without underlying malignancies. To date, reports focusing on patients with malignancies and ascites are less. The aim of the present study was to determine the prevalence and causative agents of SBP in cancer and non cancer patients and the antibacterial susceptibilities of these microorganisms. Also, to identify differences, if any, in polymorphonuclear (PMN) cell count, interleukin (IL)-6 concentration, and different biochemical diagnostic parameters, we analyzed for infected and non infected ascites in both groups.

Methods and Findings: Eighty seven hospitalized patients (29 non cancer and 58 patients with malignant tumors) with ascites were prospectively studied. Ascitic fluid was subjected to conventional culture and culture in blood culture bottles, cytological and biochemical examination. SBP was detected in both cancer and non cancer patients (37.9% & 20.7% respectively) of all age range, with monomicrobial infection (no anaerobes) in non cancer patients while it was mixed aerobic and anaerobic infection in 22.73 % of the cancer patients. Considerable differences in the antibiograms performed for the causative agents of both groups were determined. PMN count was significantly different between sterile and infected ascitic fluid in both non cancer and cancer patients. Whereas, glucose concentration was significantly different between sterile and SBP among cancer patients only. The mean value of total protein concentration in sterile malignant ascitic fluid was significantly higher than non cancer group. LDH level was found higher in sterile malignant ascites than non malignant (mean=2162.66±1620 and 216.55±163.41 respectively) with difference that was detected between sterile ascites and SBP both in cancer and non cancer patients. IL-6 concentration was found higher in cancer patients while, significant differences were found between sterile and SBP among non cancer group only.

Conclusions: The causative agents of SBP and their susceptibility patterns were completely different in patients with and without underlying malignancies. In case of diagnostic -doubts to conditions, IL-6 and some biochemical parameters as LDH and glucose of ascitic fluid may be used as rapid markers for predicting SBP.

Keywords: Ascitic fluid; Spontaneous bacterial peritonitis; Diagnostic parameters; Interleukin-6; Antibacterial susceptibility.

Introduction

Ascites is the pathologic accumulation of fluid in the peritoneal cavity as a consequence of cancer and liver diseases. It presents a difficult clinical problem causing discomfort and distress to many patients, also as more fluid accumulates it may spread up into the chest cavity (pleural effusion) and cause difficulty breathing. Bacterial infections are frequently observed com-

plications arising in patients with cirrhosis and ascites. Among these, SBP is probably the most relevant associated with high morbidity and mortality [1]. Hence, prompt detection and treatment are highly recommended [2]. The accepted pathogenic theory of SBP postulates that bacteria of enteric origin cross the intestinal wall to reach the mesenteric lymph nodes in a process called bacterial translocation (BT), and from there reach the systemic circulation [3]. The risk of developing SBP increases with worsening liver function. A first episode of SBP heralds shortened long-term survival, principally due to a 70% recurrence rate [4]. Organisms multiply in ascites only when ascites provides a good medium for growth. The proteins of the complement cascade have been found in peritoneal fluid, with lower levels in cirrhotic patients than in patients with ascites of other etiologies. Because the opsonic and phagocytic properties of PMNs are diminished in patients with severe liver disease who have lower protein content, SBP is more common with advanced liver disease [3]. Older studies reported very high (80%-100%) lethality due to SBP [5] but better results as reported in later studies [6] are, attributed to a certain extent, due to early diagnosis and treatment. However, lethality has not decreased over recent years [7, 8]. The diagnosis is established by a positive ascitic fluid bacterial culture and an elevated absolute PMN count (≥250cells/ml). Although the combination of an elevated PMN count and the yield of cultures of the ascitic fluid are considered the gold standard for the diagnosis of SBP, it has some shortcomings. First, the results of ascitic fluid culture are not readily available, delaying the diagnosis and treatment of the infection. Second, one of the most frequent variants of ascitic fluid infection is culture-negative neutrocytic ascites, which occurs in approximately 30% to 50% of patients [9 – 11].

Although SBP occurs most commonly in conjunction with cirrhosis of the liver and ascites, it is sometimes reported in patients with ascites from other causes such as nephrotic syndrome, systemic lupus erythematosus, or malignancies [12]. Malignant ascites is defined as abnormal accumulation of fluid in the peritoneal cavity as a consequence of cancer and presents a difficult clinical problem in the advanced stages of the disease. It accounts for around 10% of all cases of ascites and occurs in association with a variety of neoplasms, especially breast, bronchus, ovary, stomach, pancreas and colon cancer. Up to 20% of all patients with malignant ascites have tumors of unknown primary origin [13]. As we have little control over the underlying malignancy, only early detection and appropriate management of infectious processes can be expected to improve outcome. Diagnosis of these conditions can be difficult as there are no typical signs and symptoms, and may be revealed only if the ascitic fluid is subjected to cytological examination and cultured in blood culture bottles.

Since infections of the ascitic fluid in patients with ascites are associated with great risks and require special attention from the clinician. Therefore, it is important to investigate the pattern of infections to draw conclusion that may influence treatment regimens. Taking these facts into consideration, the aim of the present study was to determine the prevalence of spontaneous bacterial peritonitis, causative microorganisms and their antibacterial susceptibility in patients with and without underlying malignancies and ascites. Also to determine if there are differences in polymorphonuclear cell count, interleukin (IL)-6 concentration and different biochemical diagnostic parameters of infected and non infected ascitis in both malignant and non malignant patients.

Patients and Methods

This study comprised of 87 patients (58 of them were cancer patients) with ascites, hospitalized during the period between 2009 and 2010 and whose diagnoses were established by physical and ultrasound examination. Patient consent was obtained before collection of specimens. Specimens were collected from the National Cancer Institute and from Prof. Ghaffar Charity Center for Liver Diseases and Researches, Cairo, Egypt. Before any therapeutic intervention, diagnostic paracentesis of abdomen was done. Patients with recent antibacterial therapy (previous 2 weeks) and with gastrointestinal hemorrhage and under treatment with corticosteroids were excluded. Ascitic fluid specimens were subjected to conventional culture and culture in blood culture bottles, analyzed with standard biochemical methods for total protein, albumin concentration, glucose & LDH and also examined for PMN count and IL-6 concentration. The serum-ascites albumin gradient (SAAG) is defined as the difference between the serum albumin and ascites albumin concentration [2]. Statistical analysis by SAS (SAS Institute Inc., Cary, NC 27512-8000, U.S.A) was performed and the results were expressed as mean ± standard deviation.

Conventional culture method

Ten milliliters of ascitic fluid (obtained by paracentesis) were inoculated at the bedside in a blood culture bottle (BACTEC, PEDS PLUS/F medium, Becton, Dickinson Co. USA) using the BACTEC 9240 system (Becton, Dickinson Co. USA). As a positive sign appeared (according to manufacturer's instructions), a portion of the sample was chosen and subculture was performed. Also, 5 milliliters of the ascitic fluid were inoculated to thioglycollate tube medium and incubated for seven days anaerobically at 37°C (Gaspak system, BBL). The thioglycollate tubes were checked every 2 days for growth, then, once growth was detected anaerobic bacteria were isolated on Colombia Blood Agar (CBA) plates supplemented with vitamin K_1, Hemein and 5% sheep blood. The CBA plates were incubated for three days anaerobically at 37°C (Gaspak system, BBL). The MicroScan WalkAway-96 SI System (Dade Behring, West Sacramento, USA), was used for the identification of the isolated gram-negative, gram-positive and anaerobic strains using the MicroScan Dried Gram Negative ID Type 2 (Neg ID Type 2), MicroScan Dried Gram Positive ID Type 2 (Pos ID Type 2) and Microscan Rapid Anaerobe IDentification (RAID) Panels respectively.

Antimicrobial susceptibility testing of isolates

Antibiogram was performed. Both manual (disk diffusion method -Kirby-Bauer method) [14] and automated methods (MicroScan WalkAway-96 SI System, Dade Behring, West Sacramento, USA) were used to detect antimicrobial susceptibility pattern of each isolate.

Determination of PMN cells in the ascitic fluid

PMN cell count was performed by a hematological method with a light microscope in a manual counting chamber. For this method, 10 ml of ascitic fluid were collected in tubes containing ethylene diamine tetra acetic acid (EDTA) and centrifuged at 1500 r/min for 10 min. Supernatant were discarded and 40 µl of the remaining ascitic fluid were diluted with 800 µl of Turk's fluid, gently shaken and used to fill the counting chamber. The cells were counted (40 × objective) in one of the nine large squares and the number of white blood cells per cubic millimeter was calculated.

IL-6 Measurements

The IL-6 levels in the ascitic fluids were determined by using enzyme-linked immunoadsorbent assays (Human IL-6 ELISA Kit, Diaclone, France), according to the manufacturers' instructions. Samples and standard were assayed in duplicate.

Results and discussion

This study comprised of 87 patients with ascites between 41 to 62 years age group (59.8% male and 40.2% female). Fifty-eight patients (66.7%) had ascites caused by a malignant neoplasm. Among them 28 were male and 30 females, and their mean age was 52 years. The site of origin and type of tumor in patients with malignant ascites were: malignant gynecologic tumors (most common) in 22 (37.9%), carcinoma of stomach with liver metastases in 7 (12.1%), peritoneal carcinomatosis in 6 (10.3%), carcinoma of pancreas in 4 (6.9%), carcinoma of breast with liver metastases in 5 (8.6), carcinoma of lung in 4 (6.9%), non Hodgkins lymphoma in 4 patient (6.9%), colo-rectal carcinoma in 3 patients (5.2%) and three patients had 'mixed' ascites (5.2).

Microbiological examination of the ascitic fluid

Ascitic fluid culture was positive (SBP) in 28 (32.2%) of the 87 examined specimens (**Table 1**). This is in concordance to other studies that revealed SBP prevalence of 10-30% in patients with ascites admitted to hospital [9, 15-17]. SBP occurred in 22 of 58 (37.9%) cancer patients and in 6 of 29 (20.7%) patients with no underlying malignancies. SBP was detected in patients of all age range and there was no difference in mean age of infected and non infected groups (Mean 57.63±6.89 and 54±1.2 respectively). Mixed aerobic and anaerobic infections were detected in five cancer patients (22.73%). While, similar to other studies [18-20] infection was monomicrobial in case of non-cancer patients. *Escherichia coli*, that may be through translocation from the intestinal lumen, was found to be responsible for SBP cases in the non cancer patients group whereas, coagulase negative staphylococci (CNS) were the predominant species in cancer patients with ascites, (Table 1).

TABLE 1. Distribution of different microorganisms in patients with SBP.

Organism	Non Cancer patients (n=6) Number (%)	Cancer patients (n=22) Number (%)
Escherichia coli	5 (83.3)	-
Klebsiella pneumoniae	-	2 (9.1)
Pseudomonas aeruginosa	-	3 (13.6)
Staphylococcus aureus	1 (16.7)	1 (4.5)
Staphylococcus epidermidis	-	6 (27.3)
Staphylococcus hominis	-	2 (9.1)
Staphylococcus hycus	-	5 (22.7)
Bacillus	-	1(4.5)
Actinomyces israelli	-	3 (13.6)
Bacteroides distasonic	-	4 (18.2)
Total number of isolates	6	27

Runyon *et al.* & Cholongitas *et al.* [21, 22] reported similar result that most cases of SBP are due to gut bacteria such as *Escherichia coli* and *Klebsiella* and infrequently, staphylococcal infection. Species usually considered to be relatively innocuous have been found to cause serious infections in patients with underlying malignancies. Although only a few species and genera of intestinal bacteria have been proposed to translocate into the mesenteric lymph nodes, more than 70 different microbial species have been isolated in ascitic fluid from patients with bacteriologically confirmed SBP [23]. In one study, *S. aureus* was the second most commonly isolated organism in patients with decompensated cirrhosis and ascites after *E. coli* [24]. Since the proportion of isolated Gram-positive bacteria (especially coagulase negative staphylococci) increased significantly and were responsible for the majority of our culture-positive SBP cases in cancer patients besides, none of our 58 cancer patients had been receiving prophylactic antibiotic therapy, these epidemiological changes with the increasing involvement of Gram-positive bacteria might be associated with increasing frequency of invasive procedures or hospitalization which promote the prevalence of Gram-positive bacteria carriage and increase the incidence of infections caused by these microbial strains [25]. There is growing evidence by many studies that the detection of *Staphylococcus* spp. in the ascitic fluid is not because of sample contamination but because of bacterial translocation [26, 27].

Anaerobic organisms were isolated from cancer patients but not from infected ascitic fluid in non cancer patients, probably

TABLE 2A. Antimicrobial susceptibility of Gram negative bacteria isolated from ascitic fluid.

K. pneumoniae (Cancer patients) n=2(%)			*Ps. aeruginosa* (Cancer patients) n=3(%)			*E.coli* (Non Cancer patients) n=5(%)				
R	I	S	R	I	S	R	I	S	Breakpoint	Antibiotic
		2 (100%)	3 (100%)			1 (20%)	2 (40%)	2 (40%)	32	Amikacin
2 (100%)			3 (100%)			5 (100%)			16/8	Amoxicillin/K clavulanate
2 (100%)			3 (100%)			5 (100%)			16	Ampicillin
2 (100%)			3 (100%)			5 (100%)			16/8	Ampicillin/ sulbactam
2 (100%)			3 (100%)			3 (60%)		2 (40%)	16	Aztereonam
		2 (100%)	3 (100%)			5 (100%)			16	Cefazolin
		2 (100%)	3 (100%)			2 (40%)	1(20%)	2 (40%)	16	Cefepime
		2 (100%)	3 (100%)			2 (40%)	1(20%)	2 (40%)	32	Cefoperazone
		2 (100%)	3 (100%)			3 (60%)		2 (40%)	32	Cefotaxime
		2 (100%)	3 (100%)					5 (100%)	32	Cefotetan
		2 (100%)	3 (100%)			2 (40%)	2 (40%)	1 (20%)	16	Cefoxitin
		2 (100%)	3 (100%)			3 (60%)		2 (40%)	16	Ceftazidime
		2 (100%)	3 (100%)			2 (40%)	1 (20%)	2 (40%)	32	Ceftizoxime
		2 (100%)	3 (100%)			2 (40%)	2 (40%)	1 (20%)	16	Ceftriaxone
		2 (100%)	3 (100%)			2 (40%)	2 (40%)	1 (20%)	16	Cefuroxime
		2 (100%)	3 (100%)			3 (60%)		2 (40%)	16	Cephalothin
		2 (100%)	2(66.7%)		1(33.3%)	1 (20%)	2 (40%)	2 (40%)	2	Ciprofloxacin
		2 (100%)	3 (100%)			1 (20%)	2 (40%)	2 (40%)	4	Gatifloxacin
		2 (100%)	2(66.7%)		1(33.3%)	3 (60%)		2 (40%)	8	Gentamicin
		2 (100%)	3 (100%)					5 (100%)	8	Imipenem
		2 (100%)	2(66.7%)		1(33.3%)			5 (100%)	4	Levofloxacin
		2 (100%)	3 (100%)			1 (20%)		4 (80%)	16	Nitlimicin
		2 (100%)	3 (100%)			3 (60%)		2 (40%)	32	Piperacillin\tazo-bactam
2 (100%)			3 (100%)			5 (100%)			64	Piperacillin
		2 (100%)	3 (100%)			3 (60%)		2 (40%)	64	Ticarcillin\clavu-lanate
		2 (100%)	3 (100%)			3 (60%)	1(20%)	1 (20%)	8	Tetracycline
2 (100%)			3 (100%)			5 (100%)			64	Ticarcillin
		2 (100%)	3 (100%)			2 (40%)	2 (40%)	1 (20%)	8	Tobramycin
		2 (100%)	3 (100%)			2 (40%)	2 (40%)	1 (20%)	16	Trimethoprim\ sulphamethoxaz-ole

due to the inability of anaerobes to translocate across the gut mucosa and the relatively high PO_2 of ascites [28]. The use of cytotoxic agents and blood or stem cell transplantation to treat patients with cancers profoundly alters innate and acquired immunity such that the host becomes at high risk of infections by a broad spectrum of microorganisms. Defects in host innate immune response, such as neutropenia and disruption of the integrity of mucocutaneous barriers due to chemotherapy or intravascular devices, have long been recognized to be important risk factors for life threatening bacterial and fungal infections [29].

In this study, the efficacy of different antimicrobial agents was tested against the detected causative agents of SBP (**Tables 2a & 2b**). Gram negative isolates from non cancer patients, *E. coli*, were multidrug resistant strains to most antibiotics tested including non β-lactams. *E. coli* exhibited higher resistance (0% susceptibility) to Ampicillin, Ampicillin/Sulbactam, Cefazolin, Piperacillin and Ticarcillin. Whereas, the isolates were highly susceptible (0% resistance) to Imipenem (MIC=4) and Cefotetan (MIC=16), Levofloxacin and Nitlimicin (20% resistance). While it exhibited 60% resistance to Aztreonam, Ceftazidime, Cefotaxime, Ticarcillin/Clavulanate, Gentamicin, Piperacillin/

TABLE 2B. Antimicrobial susceptibility of Gram positive bacteria isolated from ascitic fluid.

Staphylococcus aureus ** n = 2(%)				CNS (Cancer patients)* n = 13(%)				
R	I	S	Breakpoint	R	I	S	Breakpoint	Antibiotic
1 (50%)		1 (50%)	32	8 (61.5%)	1 (7.7%)	4 (30.8%)	32	Amikacin
2 (100%)			16/8	13 (100%)			16/8	Amoxicillin/K clavulanate
2 (100%)			16	13 (100%)			16	Ampicillin
2 (100%)			16	13 (100%)			16	Ampicillin/sulbactam
		2 (100%)	4	10 (76.9%)		3 (23.1%)	2	Azithromycin
2 (100%)			32	11 (84.6%)	1 (7.7%)	1 (7.7%)	32	Cefazolin
2 (100%)			32	12 (92.3%)		1 (7.7%)	32	Cefepime
2 (100%)			32	9 (69.2%)	2 (15.4%)	2 (15.4%)	32	Cefoperazone
2 (100%)			32	12 (92.3%)	1 (7.7%)		32	Cefotaxime
2 (100%)			16	9 (69.2%)		4 (30.8%)	16	Cefotetan
2 (100%)			2	11 (84.6%)	1 (7.7%)	1 (7.7%)	2	Ceftriaxone
2(100%)			4	11 (84.6%)	1 (7.7%)	1 (7.7%)	4	Cefuroxime
2 (100%)			8	13 (100%)			8	Cephalothin
1 (50%)	1 (50%)		4	4 (30.8%)	3 (23.1%)	6 (46.2%)	4	Chloramphenicol
2 (100%)			64	7 (53.8%)		6 (46.2%)	64	Ciprofloxacin
	2 (100%)		8	9 (69.2%)	1 (7.7%)	3 (23.1%)	8	Clindamycin
		2 (100%)	4	8 (61.5%)	5 (38.5%)		16	Erythromycin
1 (50%)	1 (50%)		32	2 (15.4%)	3 (23.1%)	8 (61.5%)	32	Gatifloxacin
		2 (100%)	32	9 (69.2%)		4 (30.8%)	32	Gentamicin
2 (100%)			8	13 (100%)			8	Imipenem
1 (50%)	1 (50%)		64	4 (30.8%)		9 (69.2%)	64	Levofloxacin
		2 (100%)	32			13 (100%)	16	Linezolid
	1 (50%)	1 (50%)	16/8	2 (15.4%)	1 (7.7%)	10 (76.9%)	32	Moxifloxacin
2 (100%)			8	1 (7.7%)	4 (30.8%)	8 (61.5%)	64	Ofloxacin
2 (100%)			2	13 (100%)			32	Oxacillin
2 (100%)			32	13 (100%)			32	Penicillin
		2 (100%)	64			13 (100%)	8	Rifampin
	1 (50%)	1 (50%)	16	6 (46.2%)		7 (53.8%)	64	Sulfamethoxazole/ trimethoprim
		2 (100%)	16			13 (100%)	16	Tetracycline
	1 (50%)	1 (50%)	16	11 (84.6%)	1 (7.7%)	1 (7.7%)	16	Tobramycin
		2 (100%)	32			13 (100%)	32	Vancomycin
	1 (50%)	1 (50%)	64			13 (100%)	64	Synercid

Tazobactam. It should be noted that the use of Tazobactam (b-lactamase inhibitor) enhanced the activity of Piperacillin against *E. coli*. Similarly, the use of Clavulanate restored the activity of Ticarcillin against this species. In cancer patients, *Ps. aeruginosa* isolates were resistant to all tested antibacterial agents, except one isolate show susceptibility to very few agents; Ciprofloxacin (MIC=1), Gentamicin (MIC=4) and Levofloxacin (MIC=1). Controversially, *K. pneumoniae* showed higher susceptibility to the most tested antibacterial agents but exhibited resistance to some agents; Ampicillin (MIC >16), Aztreonam (MIC >16), Piperacillin (MIC >64), Ticarcillin (MIC >16). CNS were the most predominant bacteria isolated from cancer patients, while only one *S. aureus* isolate was isolated from non cancer patients. Both CNS and *S. aureus* (from cancer and non cancer patients) exhibited higher resistance to most antimicrobial agents (Amoxicillin/Clavulanate, Ampicil-

lin/Sulbactam, Ampicillin, Cefazolin, Cefepime, Cefotaxime, Ceftriaxone, Cephalothin, Imipenem, Oxacillin and Penicillin). However, they were still susceptible to few other antimicrobial agents such as Tetracycline (MIC=4), Rifampin (MIC=1), Linezolid (MIC=4), Synercid (MIC=1) and Vancomycin (MIC=2). *S. aureus* was sensitive also to Gentamicin (MIC=4), Erythromycin (MIC=0.5), Azithromycin (MIC=2), while CNS show little susceptibility to Azithromycin, Clindamycin and Gentamicin. Without early antibiotic treatment, SBP is associated with a 20–40% mortality rate [21] and death can occur in a matter of hours. Empirical antibiotic therapy must be initiated immediately after the diagnosis of the infection is made. Since the causative agent and susceptibility pattern differ in cancer patients than non cancer patients attention must be paid to the initial empirical antibiotic therapy used to cover these organisms.

Polymorphonuclears Leukocyte

There was a significant difference in PMN count between sterile and infected ascitic fluid in both non cancer and cancer patients; (P value is 0.0258 and 0.0346 respectively). An absolute peritoneal fluid neutrophil count ≥250 cells/mm^3 was reported by many as an accepted criterion for the diagnosis for SBP [9, 30, 31]. Antibiotics should be considered in any patient with a PMN count of 250 cells/mm^3 [2]. Not only does the polymorphonuclear count seem to be accurate enough to determine which patients need antibiotic therapy but it has also been considered the easiest way to establish the diagnosis of SBP [32].

Ascites biochemical characteristics

Glucose: In previous studies, little attention has been paid to the estimation of glucose in the ascitic fluid for the diagnostic purposes. In the present study there was a significant difference in glucose concentration between sterile and SBP among cancer patients (P= 0.0258). Although the glucose levels in ascites of non cancer patients were not significantly different, the mean concentration was lower in patients with SBP (**Table 3**). The glucose concentration in ascitic fluid is determined by an equilibrium between the plasma glucose level, the amount of fluid, the transport of glucose across the endothelium, and the metabolism of glucose by WBC and bacteria in the fluid. The ascitic fluid glucose concentration is similar to that of serum unless glucose is consumed by ascitic fluid bacteria, leukocytes or malignant cells. In patients with malignant ascites glucose levels in ascitic fluid were mostly lower than those in serum [28].

Protein: The mean value of total protein concentration in sterile malignant ascitic fluid was very close to the results of other studies [28, 33] and was significantly higher than non cancer group (mean 4.29±1.1 and 2.47±0.42 respectively, P value < 0.0001). This can be explained by the fact that in most patients with malignant ascitic fluid synthetic functions of the liver is not altered, and portal pressure is usually normal. Higher total protein concentration in malignant ascites could be explained by hypothesis of Garrison *et al.* [34]. They reported that tumor produced diffusible factors in extracellular fluid, and these factors could be responsible for the alteration of the microvascular permeability, which favor accumulation of the body cavity fluid. In the present study the concentration of protein was significantly lower in SBP in both cancer and non cancer patients (P = 0.0032 and 0.0133 respectively). These results were in consistent with Ljubieié *et al.* [35] demonstrating the importance of those factors in ascitic fluid defense against secondary bacterial infection. Many studies have shown that lower ascitic fluid protein levels which present a deficiency in the opsonic activity of the ascitic fluid and predisposing the patients to SBP [3, 36, 37].

Serum ascites albumin gradient: There was no statistical differences in SAAG between sterile and SBP in both non cancer and cancer groups in our study (P value = 0.8914 and 0.4108 respectively) [37- 39].

LDH: The ascitic fluid LDH level of sterile malignant patients was found significantly higher than non cancer patients (mean=2162.66±1620 and 216.55±163.41 respectively, P= 0.0017). This finding was in agreement with Akosy *et al.* [40]. Although Vieira *et al.* & Agarwal *et al.* [37, 38] reported that LDH was not significantly different in the SBP and non- SBP groups, in the present study LDH was lower in SBP. There were high significant differences between sterile ascites and SBP both in cancer and non cancer patients (P= 0.0066 and P=0.0389 respectively). This finding indicates that LDH may become useful in diagnosis of SBP.

TABLE 3. Different biochemical characteristics of the ascitic fluid.

	Non-cancer patients with sterile ascites Mean ± SD	Non cancer patients with SBP Mean ± SD	P-value	Cancer patients with sterile ascites Mean ± SD	Cancer patients with SBP Mean ± SD	P-value
Serum-Acitic Albumin Gradient (<1.1 g/dL)	1.65±0.315	1.628±0.316	0.8914	1.79±0.98	2.41±0.89	0.4108
Total Protein (≥ 2.5 g/dL)	2.47±0.42	1.91±0.67	0.0133	4.29±1.1	2.82±1.39	0.0032
Glucose(mg/dl)	202.83±109.48	158.4±73.4	0.3448	101.93±65.85	49.54±34.55	0.0258
LDH (≥200u/l)	216.55±163.41	93.22±18.64	0.0389	2162.66±1620	745.13±938.18	0.0066

IL-6 concentration in ascitic fluid

Interleukin-6 (IL-6) is a proinflammatory cytokine produced by several sources including endothelial cells, macrophages, and ovarian tumor cells.

In the present study the concentration of IL-6 was found to be higher in patients with underlying malignancies (**Table 4**). Esfandi *et al*., [41] reported elevated concentrations of inflammatory markers in people with cancer. Musselman *et al*. [42] attributed high levels of IL-6 in cancer patients to depression as an epiphenomenon of the cancer disease process not to the disease pathophysiology.

Elevated concentrations of the inflammatory cytokine interleukin-6 (IL-6) have been shown to be associated with shorter survival period in patients with various cancers [43]. There was a significant difference in IL-6 concentration between sterile and SBP among non cancer group (P=0.0272). Significantly enhanced levels of interleukin-6, as well as acute phase reactants in the ascitic fluid of patients with SBP were reported [44-47]. Also Rivera-Chavez *et al*., [48] found high levels of anti-inflammatory mediators such as IL-6, and IL-4, in peritoneal fluids of patients with pathologically proven appendicitis. These changes in cytokine levels suggest that immunological responses were caused by the infectious agents. In this study, IL-6 in SBP group was found high. Goral *et al*., [39] found high level of IL6 in SBP and reported that IL-6 levels in group with SBP began to decrease 48 hours after antibiotic treatment. In Cancer patients mean concentration of IL-6 was higher in patients with SBP than sterile ascites (Table 3) although there is no statistical differences between sterile and SBP in those patients (P=0.0733). These results could be attributed to that cancer patients have elevated cytokines concentration even without SBP. Andus *et al*., [49] results indicate that even in the absence of infection IL-6 is produced in high amounts in the peritoneal cavity of patients with hepatic or malignant ascites.

TABLE 4. IL-6 concentration in ascitic fluid.

Group of patients	SBP (n 28), Mean ± SD (pg/ml)	Sterile ascites (n 59) Mean ± SD (pg/ml)	P-value
Cancer	179.35±68.25	127±17.55	0.0733
Non cancer	162.99±53.27	94±48.16	0.0272

Conclusions

Microbiological examination of ascitic fluid is of special importance in ascites to confirm eventual spontaneous bacterial infection. We concluded that causative agents of SBP completely different in patients with and without malignancies. This should be taken into consideration when deciding the empirical antibiotic therapy that initiated before the results of ascitic fluid cultures to cover the most commonly isolated microbial organisms. Biochemical parameters and IL-6 concentration in ascitic fluid as well as PMN cell count, which, used alone or in combination, can help in differential diagnosis of ascites and so could contribute to an improvement in the survival of these patients. Some biochemical parameters as LDH and glucose of ascitic fluid may be helpful in diagnosis of SBP in case of cancer patients and need further investigation. IL-6 may be used as a rapid marker for the diagnosis of SBP in non cancer patients.

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