



Biochemical, cytological and microbiological characteristics of the cirrhotic, malignant and "mixed" ascites

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BACKGROUND: The aim of this study is to determine the value of some biochemical, cytological and microbiological analysis of ascitic fluid and serum that, used alone or in combination, can help in differential diagnosis of ascites.

METHODS: Ninety patients with ascites, hospitalized during the period between 1995 and 1998, were prospectively studied. In 56 patients liver cirrhosis, 27 patients malignant tumors, and in 7 patients liver cirrhosis plus hepatocellular carcinoma were the causes of ascites.

RESULTS: The average glucose, total protein and cholesterol concentration of the ascitic fluid and serum of patients with liver cirrhosis, malignant tumors and liver cirrhosis plus hepatocellular carcinoma were retrospectively: glucose [(6.24; 6.08), (5.25; 5.99), (7.65; 6.16) mmol/l], total protein [(16.71; 59.89), (36.12; 60.69), (17.08; 66.57 g/l)], cholesterol [(1.16; 4.87), (2.85; 5.27), (1.41; 5.54 mmol/l)], and ascitic fluid/serum ratio [(1.02; 0.27; 0.23), (0.87; 0.59; 0.54), (1.24; 0.25; 0.25)]. The mean value of serum ascites albumin gradient in these three groups of patients was respectively: [22.57, 10.97, 23.21 g/l]. The concentration of the total proteins and cholesterol were significantly higher in malignant than in cirrhotic ascitic fluid, ($p < 0.001$). In the group of patients with malignant ascitic fluid, in 33,33% of patients we found malignant cells in ascites. Spontaneous bacterial peritonitis was detected only in a group with liver cirrhosis in case of 10 patients (17.86%) with single type of organism in all.

CONCLUSION: Our study confirmed that biochemical, cytological and microbiological examinations are very helpful in differential diagnosis of ascites.

KEY WORDS: Ascites; Ascitic Fluid; Liver Cirrhosis; Neoplasms; Peritonitis

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INTRODUCTION

Many diseases are complicated by the accumulation of free fluid within the peritoneal cavity i.e. the onset of ascites. The most common cause of ascites is liver cirrhosis, but in about 20 percent of cases there is an extrahepatic cause. On the basis of their comprehensive study Runyon and colleagues report that

parenchymal liver disease are the most common cause in about 80%, and then malignancy 10%, heart failure 5%, tuberculosis 2%, and other causes in the rest 3% of cases (1).

Although the diagnosis of ascites on the basis of physical examination is easy, the etiologic diagnosis is difficult and sometimes is an insoluble problem for clinicians. The diagnosis of the cause of ascites formation is based on the results of the history, physical examination, and ascitic fluid analysis. Sometimes few other tests are required. All diseases, which are complicated with the onset of ascites, can be divided into two groups: with portal hypertension as a main pathophysiological mechanism, and without portal hypertension, but with peritoneal chafe. Many studies were concerned with the analysis of ascitic fluid and serum and the problem of differential diagnosis of ascites tried to discover and define some reliable parameters, most often biochemical (2,9,13,16). There were biochemical parameters at first time

Abbreviations:

AF - ascitic fluid; S - serum; SAAG - serum ascites albumin gradient; SBP - spontaneous bacterial peritonitis; SCA - sterile cirrhotic ascites

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accepted, and then refused as uncertain. It was necessary to find out new, more valuable, biochemical parameters. The serum-ascites albumin gradient is one of them. The cytological diagnosis of ascitic fluid has been discussed in medical literature for almost a hundred years. Cytologic examination of ascitic fluid has increasingly gained acceptance in clinical medicine, to such an extent that a positive diagnosis is often considered as a definitive test. Relatively frequent involvement of microorganisms in pathogenesis of ascites (primary or secondary peritonitis), or as a cause of complications in patients with chronic liver disease and ascites, require careful microbiological examination of ascitic fluid. Microbiological examination of ascitic fluid is of special importance in cirrhotic ascites to confirm eventual spontaneous bacterial infection, i.e. spontaneous bacterial peritonitis.

The aim of this study was to determine the diagnostic value of some biochemical parameters in ascitic fluid as well as in the serum, cytologic and microbiologic analysis of ascitic fluid, which, used alone or in combination, can help in differential diagnosis of ascites. Our aim was also to determine the biochemical, cytological and microbiological characteristics of the cirrhotic, malignant and 'mixed' ascites.

PATIENTS AND METHODS

Ninety patients with ascites, 51 male and 39 female, ranged in age from 29 to 80 years, mean age 61 year, hospitalized in the Clinic of Gastroenterology and Hepatology, Clinical Center, University of Niš during the period between 1995 and 1998, were prospectively studied.

After the clinical examination patients undergone the laboratory and instrumental methods, selectively applied, depended on symptoms and signs of disease.

The imaging studies (radionuclide liver spleen scan, ultrasound, and/or computerized tomographic scan) were performed to assess the cause of ascites formation. Laparotomy or autopsy determined the localization of tumor, and for patients who did not undergo one of these procedures, a judgment was made as to localization of tumor and pathophysiology based on the imaging modalities. The diagnosis of cirrhosis was established on the basis of the clinical examination, biochemical test and instrumental examination and/or liver biopsy.

Abdominal paracentesis was performed in the first 24 hours after admission of patients, using aseptic technique, with the 22 gauge needle, in the left lower abdominal quadrant, on the line between umbilicus and anterior superior iliac spine, at the point between the outer and middle third. The samples of ascitic fluid were immediately conducted to biochemical, cytological and microbiological laboratory to be analyzed. Blood samples were taken for simultaneous determinations of some biochemical parameters.

Ascitic fluid and blood were examined for glucose, protein, albumin and cholesterol concentration, with standard biochemical methods. The serum-ascites albumin concentration gradient (SAAG) is defined as the difference between the serum albumin and ascites albumin concentration. Results are expressed as mean $\pm$ standard deviation. The Student's t-test and Mann-Whitney Rank Sum test were used for statistical analysis of the data.

Smears of ascitic fluid were fixed and stained with Hematoxylin-Eosin and Papanicolaou and examined microscopically for their cellular content.

Aerobic and anaerobic cultures were performed. Specimens were plated on blood agar, chocolate agar, endo agar and glucose broth, for isolation of aerobic bacteria and Sedler substratum and tioglicolat for the isolation of anaerobic bacteria. Api 20A and VITEC system for rapid bacteria identification identified the isolated bacteria with standard microbiological methodology. The sediment from 40 ml. of ascitic fluid was cultured for mycobacteria. The ascitic fluid white blood cell and neutrophil counts were determined on the basis of morphologic appearance in a Manuel counting chamber.

RESULTS

After the examinations, the patients were divided into three groups (1) patients with cirrhotic ascites, (2) patients with malignant ascites and (3) with mixed ascites. After the microbiologic examination patients with cirrhotic ascites were divided into two subgroups (a) with sterile cirrhotic ascites and (b) with spontaneous bacterial peritonitis.

Fifty-six patients (62.22%), had liver cirrhosis. Among the cirrhotic patients 38 were male and 18 females, and their mean age was 60 years. The cause of cirrhosis was alcohol abuse in 31, HBV infection in 10, HCV in 8 and unknown in 7 patients.

Twenty-seven patients (30%) had ascites caused by a malignant neoplasm. Among them nine were male and 18 females, and their mean age was 64 years.

The site of origin and type of tumor in patients with malignant ascites were: malignant gynecologic tumors (most common), in 10 (37.03%), carcinoma of stomach with liver metastases in 4 (14.81%), peritoneal carcinomatosis in 4 (14.81%), carcinoma of pancreas in 3 (11.11%), carcinoma of breast with massive liver metastases in 2 (7.4%), massive liver metastases alone in 2 (7.4%), carcinoma of lung in 1 (3.7%), non Hodgkin's lymphoma in 1 patient (3.7%).

Seven patients (7.78%), had 'mixed' ascites, in all cases caused by hepatocellular carcinoma superimposed on cirrhotic liver. Four were males and three females with mean age 61 year.

Biochemical analysis of ascitic fluid. The average glucose, total

protein and cholesterol concentration of the ascitic fluid and serum, and the mean ascitic fluid-serum ratio of patients with liver cirrhosis, malignant tumors and liver cirrhosis plus hepatocellular carcinoma were: glucose [(6.24±1.97; 6.08±2.2), (5.25±1.8; 5.99±3.28), (7.65±4.83; 6.16±3.71) mmol/l], total protein [(16.71±11.93; 59.89±10.09), (36.12±15.46; 60.69±9.5 g/l), (17.08±6.81; 66.57±6.24g/l)g/l], cholesterol [(1.16±1.14; 4.87±2.50), (2.85±1.46; 5.27±3.06), (1.41±0.49; 5.54±2.9 mmol/l)] respectively.

The mean value of SAAG in these three group of patients was respectively: [(22.57±8.11, 10.97±7.43, 23.21±6.55 g/l)].

The ascitic fluid/serum ratio of glucose, protein and cholesterol, in these three group of patients was [(1.02; 0.27; 0.23), (0.87;

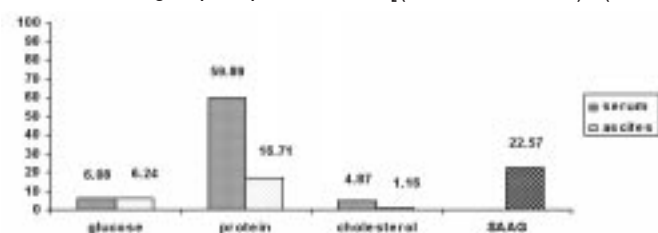


Figure 1. Ascitic fluid and serum concentrations of glucose, total protein, cholesterol and serum ascites albumin gradient in patients with liver cirrhosis

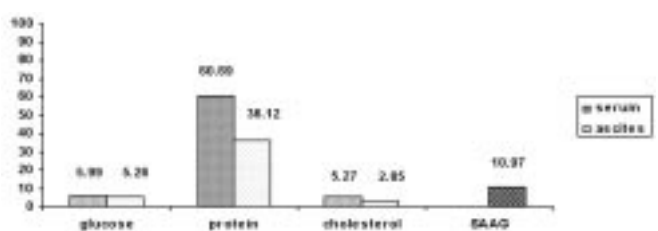


Figure 2. Ascitic fluid and serum concentrations of glucose, total protein, cholesterol and serum ascites albumin gradient in patients with malignant neoplasms

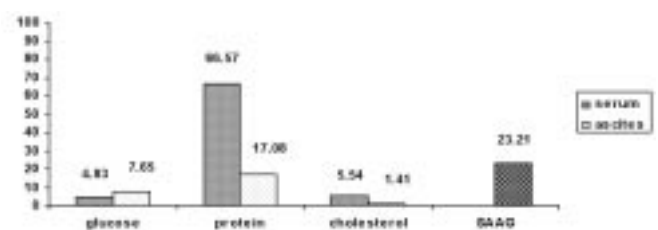


Figure 3. Ascitic fluid and serum concentrations of glucose, total protein, cholesterol and serum ascites albumin gradient in patients with liver cirrhosis plus hepatocellular carcinoma

0.59; 0.54), (1.24; 0.25; 0.25)], respectively (Figure 1,2,3).

Correlating the concentrations of glucose between the cirrhotic and malignant ascites no statistically significant differences of glucose concentration ($p>0.05$) was found.

Ascitic fluid total protein and cholesterol concentrations were significantly higher in malignant than in cirrhotic ascites ($p<0.001$) (Figure 4,5).

Cytologic analyses of ascitic fluid. Five types of cells were differentiated in ascitic fluid of each group of patients in variable

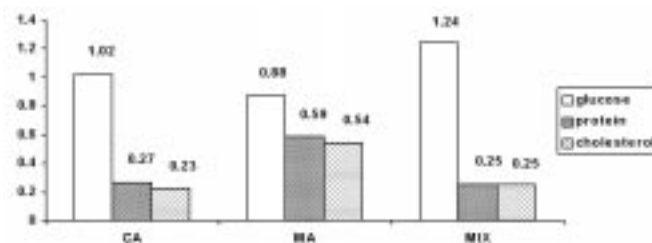


Figure 4. The ascitic fluid/serum ratio of glucose, protein and cholesterol

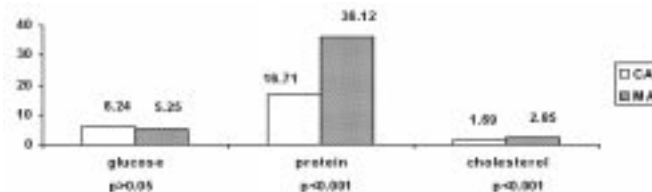


Figure 5. Mean levels of glucose, total protein and cholesterol in cirrhotic and malignant ascites

percentage.

The mesothelial cells (85.71%) and lymphocytes (78.57%), represented the two major types of cells in the cirrhotic ascites, while the erythrocytes, polymorphonuclears and macrophages represented 21.42, 26.78 and 44.64 percent of the population respectively.

In the group of patients with malignant neoplasms the mesothelial cells (77.77%) and erythrocytes (59.25%) represented the two major types of cells, while the lymphocytes, polymorphonuclears and macrophages represented 55.55, 44.44 and 14.81 per cent respectively. Malignant cells were present in 33.33% of cases, contrary to the group with 'mixed' ascites where malignant cells were not detected.

The cellular composition of 'mixed' ascites was very similar to cirrhotic ascites (Figure 6).

Microbiological examination of ascitic fluid. Aerobic and anaerobic cultures obtained in all patients were positive in 10 patients

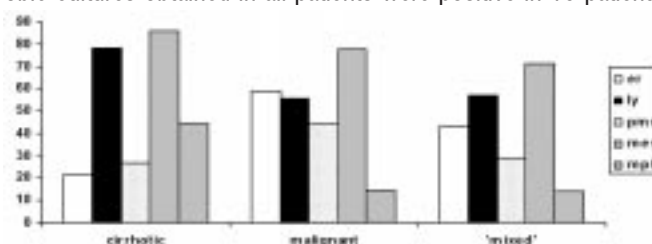


Figure 6. Citogram of cirrhotic, malignant and 'mixed' ascitic fluid

(11.11%), only in a group of patients with cirrhotic ascites. A single organism was isolated in all patients. In the group of patients with malignant and 'mixed' ascites cultures were negative in all cases. After microbiologic examination the group of patients with cirrhotic ascites was divided into two subgroups: sterile cirrhotic ascites (SCA) spontaneous bacterial peritonitis (SBP) (Table 1). The presence of *Escherichia coli* was demonstrated in 2 patients, *Staphylococcus aureus* in 4, *Pseudomonas maltophilia*,

Table 1. Frequency of spontaneous infection of ascitic fluid

Subgroup	Number	%
Sterile cirrhotic ascites (SCA)	46	82.14
Spontaneous bacterial peritonitis (SBP)	10	17.86
Total	56	100.00

Pseudomonas aeruginosa, *Staphylococcus epidermidis* and *Klebsiella*, each in one (Table 2).

In the SBP group the mean ascitic fluid neutrophil count was $404 \pm 129/\text{mm}^3$, which was significantly higher ($p < 0.001$) than

Table 2. Flora of ascitic fluid

Organism	Number	% (n=10)
<i>Escherichia coli</i>	2	20
<i>Staphylococcus aureus</i>	4	40
<i>Staphylococcus epidermidis</i>	1	10
<i>Pseudomonas aeruginosa</i>	1	10
<i>Pseudomonas maltophilia</i>	1	10
<i>Klebsiella</i>	1	10
Total	10	100

those in the sterile ascites group $39 \pm 12/\text{mm}^3$.

DISCUSSION

Biochemical, cytological and microbiological examination of ascitic fluid is of great importance for a clinician when making etiologic diagnosis of ascites. Distinction between malignant and other causes of ascites has important therapeutic and prognostic implications. Biochemical analysis of ascitic fluid can be helpful in determining the underlying disease (2-4). We discuss the diagnostic accuracy of common and newer biochemical parameters (glucose, total protein, serum-ascites albumin gradient and cholesterol), bacteriologic and cytologic examinations.

Little attention has been paid to the estimation of glucose in the ascitic fluid for the diagnostic purposes. There are only a few articles dealing with this problem. Jain et al. (5) found that in cases of tuberculous peritonitis, the ascitic fluid sugar was low compared to blood sugar. Other investigators (6,7,8) made similar observations. Gorozhanskaya et al. (9) stated that ascites from patients with malignant neoplasms had a high content of glucose. 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.

Mean values of glucose concentrations in cirrhotic and malignant ascitic fluid in this study were lower than in the study of Bar-Meir et al (3). The glucose levels in ascites and serum in the first group of patients were very similar, probably as a result of fact that glucose molecule is small enough to diffuse readily into ascites. Therefore, 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 hepatic portal hypertension the glucose levels were higher in the ascitic fluid than in serum. The other investigators also observed this phe-

nomenon (10,11). Similar results were in the group of patients with 'mixed' ascites. That was expected because of underlying cirrhosis in this group of patients. In patients with malignant ascites glucose levels in ascitic fluid were mostly lower than those in serum, similar to the results of Polak et al (11). This is probably due to high uptake of glucose by malignant cells, but only in patients with peritoneal carcinomatosis. These findings led as to conclusion that glucose content of ascitic fluid should always be compared with that of blood. Absolute value of ascitic fluid glucose is of minor significance.

Mean values of total protein concentrations in cirrhotic ascites in this study belong to the group of transudates in accordance with the limit value for differentiating the transudate and exudate of 25 g/l (12,13). But in 10.71% of patients from this group, total protein concentration was higher than 30 g/L, and in accordance with the previous classification was exudate. Similar results were observed in other studies (10,14,15). This can be explained by the fact that protein concentration in cirrhotic ascites is determined by serum protein concentration (direct correlation) and portal pressure (inverse correlation). In patients with liver cirrhosis synthetic function of the liver is insufficient and consequently followed by hypoproteinemia and filtration of smaller amount of proteins in ascitic fluid. A cirrhosis with relatively high protein concentration will have a relatively high ascitic fluid protein concentration. The mean values of total protein concentration in cirrhotic ascites in this study are close to results obtained by Wilson (10) and Scholmerich (16). The mean value of total protein concentration in malignant ascitic fluid was very close to results of other studies (2,16-18) and was significantly higher than in cirrhotic ascites. This can be explained by the fact that in patients with malignant ascitic fluid synthetic function of the liver is not altered, and portal pressure is normal except in cases with massive hepatic metastasis, because of massive hepatic replacement by tumor with concomitant portal hypertension and hypoproteinemia. Higher total protein concentration in malignant ascites could be explained by hypothesis of Garrison et al. (19) about the presence of tumor produced diffusible factors in extracellular fluid. These factors could be responsible for the alteration of the microvascular permeability, which favor accumulation of the body cavity fluid.

Due to problems with exudate-transudate concept after the 1980's serum ascites albumin gradient SAAG (defined as the serum albumin concentration minus ascitic fluid albumin concentration) has been proposed as a better physiologically based parameter for differential diagnosis of ascites (20). This "gradient" has been shown to correlate directly with portal pressure so that patients with gradients of 11 g/L or greater have portal hypertension, whereas those with gradients less than 11 g/L do not

have the disorder (21,22). Runyon (1) stated that if the SAAG is greater than 11 g/L the patient has portal hypertension with approximately 97 per cent accuracy. Present study confirmed the high sensitivity of SAAG in a group of patients with cirrhotic ascites, and lower sensitivity in a group of patients with malignant ascites. All patients with "mixed" ascites because of underlying cirrhosis had SAAG greater than 11 g/L. This study also confirmed that SAAG has no provision for patients with two causes for ascites formation (i.e. "mixed" ascites). Usually, these patients have cirrhosis plus another cause such as peritoneal carcinomatosis, peritoneal tuberculosis or hepatocellular carcinoma, and SAAG higher than 11 g/L.

Interesting finding of this study is that significant number of patients (37.04%) with malignant ascites, in contrast with expectations, had SAAG greater than 11 g/L. Most of them had massive hepatic metastases, and as a consequence portal hypertension. Our results suggest that the serum ascites albumin gradient is more useful than the ascitic fluid total protein concentration as a marker for portal hypertension, but have no benefits in patients with malignant ascites caused by massive hepatic metastasis.

Recently, cholesterol determination has been proposed as a useful test in detecting malignant ascites. Some investigators found that cholesterol concentration is significantly higher in malignant ascites than in the cirrhotic one (18,23).

Comparing the ascitic fluid cholesterol levels in malignant and cirrhotic ascites Giannoulis (24) and Bansal (25) stated that cholesterol level in the ascitic fluid is a useful and sensitive biochemical marker which enhance the diagnostic yield in the evaluation of ascites. In order to compare the diagnostic value of cholesterol in the differentiation between malignant and cirrhotic ascites, with application of the cutoff concentrations given in the literature (1.25mmol/L) the high sensitivity and specificity of cholesterol is revealed (18). Xie (26) found that ascitic fluid cholesterol concentrations were increased 3.9 times in patients with peritoneal carcinomatosis as compared to liver cirrhosis.

In contrast Seelis et al.(27) found that sensitivity for diagnosis of malignant ascites was 45% for ascitic fluid cholesterol (cutoff 1.25 mmol/L), and conclude that AF cholesterol is not of predictive value in the differential diagnosis of malignant and benign ascites.

Runyon (1,28) stated that measuring of ascitic fluid cholesterol is unhelpful in differential diagnosis of ascites, because many patients with massive liver metastasis do not have abnormally elevated ascitic fluid cholesterol concentration and patients with pancreatic or cardiac ascites may have false positive values.

Our results suggest that determination of ascitic fluid cholesterol is useful for differential diagnosis of ascites.

The cytological diagnosis of body fluids has been discussed in

medical literature for almost 130 years. Luecke and Klebs (29) were the first to observe malignant cells in ascitic fluid. Probably no other area of clinical cytology has as many pitfalls as can be encountered in this area. The differentiation of malignant cells from degenerated mesothelial and other non-malignant cells can be very difficult. Bakalos (30) emphasizes that by the Papanicolaou method the possibility of distinguishing normal mesothelial from neoplastic cells has greatly increased, but false positive and false negative results are not rare.

The observation of this study is that lympho-plasmacyte mononuclear cells dominate in cirrhotic ascites followed by mesothelial cells. Erythrocytes are principally absent. The cellular content of malignant and "mixed" ascites is similar to that, but erythrocytes were present in greater percentage. We also detect malignant cells but in a smaller percentage than in the earlier literature, where cytology was reported to be sensitive 58 to 75 per cent (31-38). It is possible because ascitic fluid was fixed and stained with Hematoxylin-Eosin and rarely with Papanicolaou method. In the group of patients with "mixed" ascites we did not detect malignant cells in ascitic fluid. Runyon (1) stated that cytology should be positive only when tumor cells line the peritoneal cavity (i.e. peritoneal carcinomatosis). Cytology should not be expected to detect tumor when peritoneum is not involved (e.g. hepatoma, massive liver metastasis, malignant lymphoma). He also suggested that because hepatoma rarely metastasizes to peritoneum cytology is almost never positive (28). Colli (31) found positive ascitic cytology in 12 percent of patients with hepatocellular carcinoma.

The finding of the present study is also that macrophages are present in the higher percentage in the cirrhotic than in the malignant ascites, probably as a result of the presence of some macrophage inhibiting factors in the malignancy related ascites. The light microscopy alone is not adequate method for the diagnosis of malignancy in ascitic fluid.

Spontaneous bacterial peritonitis is a common and frequently fatal complication of cirrhosis. Six to 27 percent of patients with ascites are found to have infected ascites at the time of hospital admission (39,40). Spontaneous bacterial peritonitis has been described in settings other than cirrhosis ascites (41), but it is distinctly unusual in malignant ascites.

This prospective study documents cases of SBP only in patients with liver cirrhosis. SBP was not found in other patients with ascites of other causes, which can suggest that portal hypertension, is the main condition for development of spontaneous infection of ascitic fluid. It is interesting that in the group of patients with malignant ascites were four patients with massive hepatic metastasis and portal hypertension, but ascitic fluid was sterile. Even with the large number of cancer patients with ascites, only

a rare case report has described SBP in this setting. Kurtz et al. (42) believe that spontaneous bacterial peritonitis does not occur in malignant ascites. A rare patient may develop SBP, but this represents the exception, not the rule.

Also all ascitic fluid specimens in that group of patients were sterile, although in all cases hepatoma developed on cirrhotic liver.

Most common isolated organisms from ascitic fluid in our study were gram positive aerobic organisms. Anaerobic organisms were not isolated from infected ascitic fluid, probably due to the inability of anaerobes to translocate across the gut mucosa and the relatively high PO_2 of ascites (43). Richardet et al. (44) found in their comprehensive study that most common organisms isolated from ascitic fluid in patients with spontaneous bacterial peritonitis were gram negative aerobic organisms. Anaerobic organisms were rare cause of spontaneous infection of ascitic fluid. Prevalence of spontaneous infection of ascitic fluid of 18% in our study is quite high and comparable with results of other studies (39,40,44,45).

The results of our study suggest that biochemical, microbiological and cytological analysis of ascitic fluid may help to establish the cause of ascites formation.

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