



Original Article

Ascitic fluid cytology and its implications in the clinical approach

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Abstract

Ascitis is a consequence or complication of a number of diseases. It often presents as a common diagnostic and therapeutic dilemma to the pathologists and physicians. Both non neoplastic and neoplastic causes of ascitis can be identified by the relatively non invasive technique of ascitic fluid cytology. The present study aims to assess the value of ascitic fluid cytology in the differential diagnosis of ascitis and its usefulness in the patient management.

This prospective study was conducted from June 2014 to June 2018. Total 1600 patients with ascitis were included in the study. Total cell count of ascitic fluid was done with improved Neubauers counting chamber. Smears were studied for cell type and cellular features.

Most common cause of ascitis was cirrhosis [80.7%]. Malignant ascitis was noted in 4.5% cases. It was mostly (98.6%) due to metastasis from ovarian, gastrointestinal, unknown primary and pancreatic malignancies. The conditions like spontaneous bacterial peritonitis, eosinophilic ascitis and filarial ascitis were readily diagnosed by total cell count and ascitic fluid cytology. Ascitic fluid cytology was useful not only in the diagnosis but also to assess the response to treatment in these cases.

The careful cytomorphological examination of ascitic fluid is a valuable, simple, rapid, inexpensive and reliable technique in the differential diagnosis of ascitis, particularly in resource limited settings.

Keywords: Ascitic fluid cytology, ascitis, cirrhosis, spontaneous bacterial peritonitis.

Introduction

Ascitis is the pathological accumulation of fluid within the peritoneal cavity. Clinically, ascitis is a consequence or complication of a number of diseases; including hepatic, cardiac and renal diseases, infections and malignancy. ^[1] It often

presents as a common diagnostic and therapeutic dilemma to the pathologists and physicians. The proper evaluation of ascitic fluid helps in narrowing the diagnostic dilemma faced by the physicians and helps in better management of the patients. ^[2]

The cytological interpretation of individual cells that are exfoliated in the fluid is of paramount

importance since they provide an insight into the diagnostic, prognostic and therapeutic aspect of various pathological processes in the body. Most important goal of ascitic fluid cytology is the detection of malignant cells. But many other conditions such as inflammatory diseases, infections with bacteria, fungi, viruses and parasitic infestations can also be identified^[3].

The present study aims to assess the value of ascitic fluid cytology in the differential diagnosis of ascitis and its usefulness in the patient management.

Materials and Methods

This prospective study was conducted in the Department of Pathology, in a tertiary health care centre over a period of 4 years. The study included 1600 patients who presented with ascitis in the Department of Gastroenterology. Relevant clinical information regarding age, sex and symptoms was obtained.

Majority of the samples were processed immediately. But in some cases, if there was delay, samples were stored in refrigerator at 4 °C. Gross appearance of the fluid was noted. The fluid was divided in two parts. One part was used for cell count by improved Neubauer counting chamber. The other part was poured in the centrifuge tubes and centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded. Part of sediment was transferred to a clean glass slide and mixed with a drop of 1% Toluidine blue. After placing the coverslip, the slide was examined under the microscope for immediate identification of cell morphology. Remaining sediment was transferred with the help of pipette to two clean slides. One smear was air dried and stained with May Grunwald Giemsa [MGG] stain. Other smear was fixed in 95% alcohol and stained with Hematoxylin and Eosin [H&E] stain. Smears were examined for cell type and cellular features. A repeat examination of fluid was done in those cases, in which malignancy was suspected, but was not conclusive on initial examination.

Results

Total 1600 patients were studied. The age of the patient ranged from minimum of 2 years to a maximum of 86 years. The maximum number of cases was in the age group of 41-60 years [65.5%]. Male preponderance was noted with the male to female ratio of 2.3:1.

Pale and clear fluid was seen mostly in cases of cirrhosis while haemorrhagic effusion was seen in malignancy and turbid fluid was noted mostly in spontaneous bacterial peritonitis [SBP]. The most frequent cause of non neoplastic ascitis was cirrhosis with 80.7% cases [Table 1]. Tuberculous peritonitis constituted 3.7% cases. These cases were confirmed by polymerase chain reaction (PCR). Total cell count less than 100/mm³ were seen in cases of cirrhosis. High total cell counts [$> 500/\text{mm}^3$] were seen in all cases of spontaneous bacterial peritonitis [SBP] and tuberculosis [Table 2]. Neutrophilic predominance was seen in SBP [Fig 1]. Lymphocytic predominance and in few cases mesothelial predominance was seen in tuberculosis [Fig 2]. Two cases of eosinophilic and filarial ascitis were also detected [Fig 3 & 4]

The commonest malignancy metastasizing to peritoneal cavity was adenocarcinoma. [Fig 5] The distribution of various primary sites for metastatic peritoneal effusion is given in [Table 3]. The youngest patient of this study had malignant ascitis secondary to Wilm's tumor. In 8 cases, of ovarian malignancy, positive ascitic fluid cytology was the first indication of malignancy. Subsequent pelvic ultrasonography revealed an ovarian mass. A single case of primary peritoneal mesothelioma was detected on ascitic fluid cytology [Fig 6]. Confirmation was done by biopsy and immunohistochemistry.

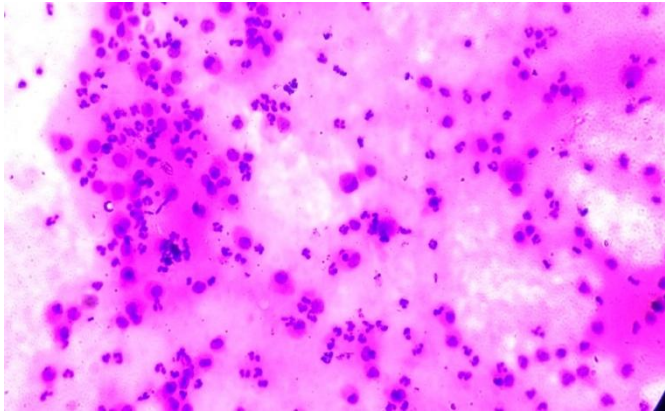


Fig 1: SBP showing numerous neutrophils. [H&E 400X]

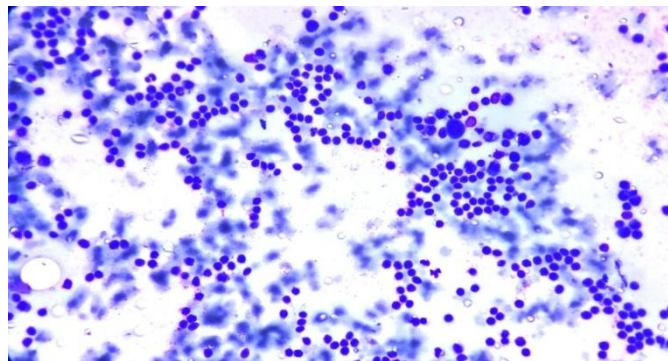


Fig 2: TB ascitis showing predominantly lymphocytes. [MGG 400X]

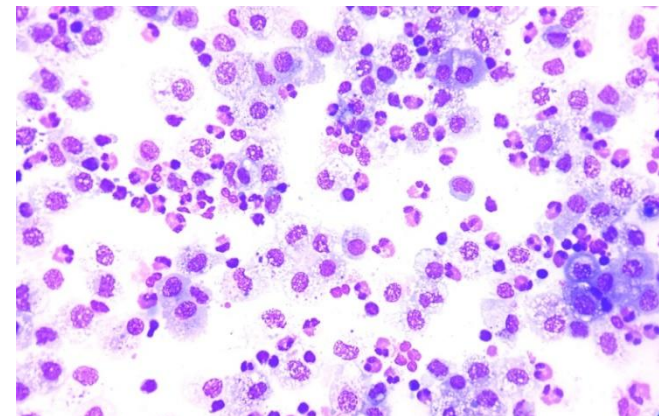


Fig 3: Eosinophilic ascitis showing predominantly eosinophils. [MGG 400X]



Fig 4: Filarial ascitis showing microfilaria. [MGG 400X]



Fig 5: Metastasis of adenocarcinoma showing 3D clusters and acinar arrangement of tumor cells. [MGG 400X]

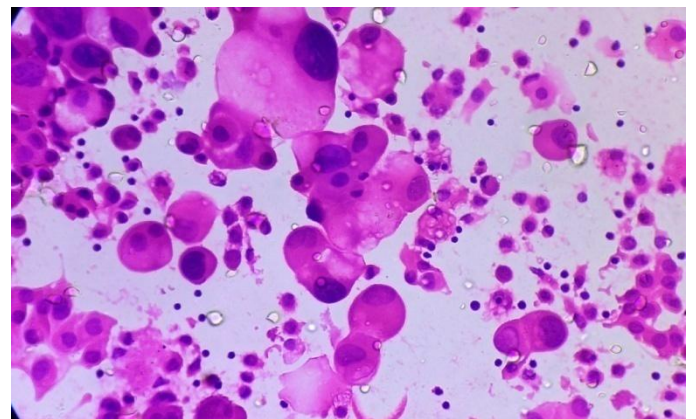


Fig 6: Mesothelioma showing cellular smears with pleomorphic mesothelial cells [H&E 400X]

Table 1: Etiological classification of ascitis cases

Causes of ascitis	No. of patients	Percentage
Liver cirrhosis	1292	80.7%
Pancreatitis	90	5.7%
SBP	82	5.2%
Malignancy	72	4.5%
TB	60	3.7%
Eosinophilic gastroenteritis	2	0.1%
Filarial	2	0.1%
Total	1600	100%

Table 2: Distribution of total cell count in ascitis cases

Diagnosis	Total cell count			No. of cases
	<100/mm ³	100-500/mm ³	>500/mm ³	
Liver cirrhosis	900	392	-	1292
Pancreatitis	15	50	25	90
SBP	-	-	82	82
Malignancy	-	32	40	72
TB	-	5	55	60
Eosinophilic GE	-	-	2	2
Filarial	-	2	-	2

Table 3: Distribution of primary sites in metastatic peritoneal effusion

Primary site	No. of cases (N=71)	Percentage
Ovary	30	42.2%
GIT	19	26.7%
Unknown	15	21.2%
Pancreas	6	8.5%
Wilms tumor kidney	1	1.4%
Total	71	100%

Discussion

The cytological examination of effusion is a complete diagnostic modality which aims at pointing out the etiology of effusion. The diagnostic performance of the cytological study of fluid may be attributable to the fact that the cell population present is representative of much larger surface area than that obtained by needle biopsy^[4]. The most useful test in establishing the differential diagnosis of ascitis is ascitic fluid cytology and ascitic fluid cell count.

In the present study, total 1600 cases of ascitis were included. This is one of the largest studies dealing only with the ascitic fluid. Other authors have also included pleural and pericardial fluids in their study^{[5]-[8]}. Males outnumbered females with M:F ratio of 2.3:1. This is in concordance with most of the studies^{[6]-[8]}. Maximum number of cases (65.5%) was in the age group of 41-60 years as observed by other authors^{[2],[7]}. Cirrhosis was the most common cause of ascitis (80.7%), which corresponds with other studies^{[1],[2],[6],[7]}.

Spontaneous bacterial peritonitis (SBP) is defined as bacterial infection of ascitic fluid and peritoneum [ascitic fluid neutrophil count $\geq 250/\text{mm}^3$] in patients with ascitis in absence of any intraabdominal source of infection or malignancy. The development of SBP is associated with poor outcome. It is recommended that all cirrhotic patients with ascitis should have routine diagnostic paracentesis irrespective of clinical suspicion of SBP^[9]. At the end of 48 hours of antibiotic treatment, if ascitic fluid neutrophil count has not decreased by at least 25% from pretreatment ascitic fluid neutrophil count, it is better to change the antibiotics based on culture and sensitivity report of ascitic fluid. Antibiotic treatment can be

discontinued after neutrophil count is $<250/\text{mm}^3$ ^[9]. Thus, cell count and ascitic fluid cytology is important in the diagnosis and also to assess the response to treatment in SBP.

Tuberculous peritonitis still continues to be a health problem in developing countries. About 73% patients present with ascitis^[10]. The ascitic fluid in tuberculosis is straw coloured with protein $>3 \text{ gm/dl}$ and total cell count of $150-4000/\text{mm}^3$ consisting predominantly of lymphocytes ($>70\%$). But neutrophilic or mesothelial predominance can also be seen. The presence of lymphocytic ascitis therefore is not a reliable marker of tuberculosis and should be considered merely as an indication for further investigations^{[10][11]}. Our findings are in agreement with other authors^{[2],[12]}.

Eosinophilic gastroenteritis is a rare and potentially fatal condition with characteristics of peripheral eosinophilia and eosinophilic infiltration of gastrointestinal tract. It is classified in 3 types- a) predominant mucosal disease b) predominant muscle layer disease c) predominant subserosal disease characterized by eosinophil rich ascitis. The mainstay of diagnosis of subserosal type is confirmation of eosinophil rich ascitic fluid and peripheral eosinophilia^{[13],[14]}. In the present cases, 2 cases had eosinophilic ascitis. The ascitic fluid total cell count was $>1000/\text{mm}^3$ with 80% eosinophils and peripheral eosinophilia. Other causes of eosinophilic ascitis like malignancy, allergic states, parasitic infestation and dialysis were excluded. Endoscopic biopsy from duodenum and colon were unremarkable. These findings confirmed the diagnosis of subserosal eosinophilic gastroenteritis. Patients were treated with corticosteroids with rapid symptomatic improvement and normalization of hypereosinophilia.

Filariasis is still endemic in many parts of India. Ascitis is a rare presentation of filariasis. Demonstration of parasites on ascitic fluid cytology is helpful in the correct diagnosis but also in instituting specific treatment^[15]. In present study, microfilaria was seen in 2 cases. Patients were given antifilarial treatment. In both the patients ascitis resolved on follow up.

Recognition of malignant cells is the most important goal of ascitic fluid cytology and is the first line of investigation in cases with suspected neoplastic effusion. A positive effusion for malignant cells is an important prognostic indicator in cancer patients. In the present study, 72 (4.5%) cases had malignant ascitis^[5]. The most common primary site was ovary followed by GIT. In case of suspicious samples, a repeat examination should be advised. The rate of detection of malignant cells increased, when multiple samples were studied. Our findings correspond with those of the other studies^{[5],[7],[12],[16]}. Only one case of primary peritoneal mesothelioma was detected on ascitic fluid cytology. The smears were highly cellular with pleomorphic mesothelial cells arranged in groups with scalloped borders, singly dispersed cells and in couplets with window formation. Individual cell showed round to oval hyperchromatic central to eccentrically placed nucleus and moderate amount of dense, eosinophilic cytoplasm. Cytoplasmic vacuolation, binucleation and multinucleation were frequently seen. Patel et al^[17] described similar findings in cytologic analysis of fluid from malignant mesothelioma. Further confirmation was done by biopsy and immunohistochemistry.

Conclusion

Both non neoplastic and neoplastic conditions causing ascitis can be diagnosed on ascitic fluid cytology. The careful cytomorphological examination of ascitic fluid is a valuable, simple, rapid, inexpensive and reliable technique in the differential diagnosis of ascitis, particularly in resource limited settings.

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