European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 9, Issue 4, Summer 2022

ORIGINAL RESEARCH

A HOSPITAL BASED OBSERVATIONAL STUDY TO CORRELATE THE CYTOLOGICAL FEATURES OF THE ASCITIC FLUID WITH CLINICAL DATA AT NEWLY ESTABLISHED TERTIARY CARE CENTER: A CLINICO-PATHOLOGICAL STUDY

[1]Anil Kumar Sethiya, [2]Madan Kumar Solanki, [3]Hanuman Ram Choudhary

[1]Assistant Professor, [3]Professor, Department of General Medicine, Government Medical College, Barmer, Rajasthan, India
[2]Assistant Professor, Department of Pathology, Government Medical College, Barmer, Rajasthan, India

Correspondence:
Madan Kumar Solanki
Assistant Professor, Department of Pathology, Government Medical College, Barmer, Rajasthan, India
E mail: dr.madansolanki@gmail.com

ABSTRACT

Background: The proper evaluation of ascitic fluid helps in narrowing the diagnostic dilemma faced by the physicians and helps in better management of the patients. Early and accurate diagnosis often depends on appropriate ascitic fluid analysis. The aim of this study to correlate the cytological features of the ascitic fluid with clinical data at newly established tertiary care center.

Materials & Methods: A prospective study was conducted in the Department of Pathology, in a tertiary health care centre over a period of one year. Cytological examination was performed to reveal important information regarding the causes of ascites and classified as benign (with or without infection/ spontaneous bacterial peritonitis), suspicious or malignant conditions. Cytomorphology of the cells was studied and documented paying attention to cellular arrangement, cytoplasmic features and nuclear characteristics. Clinical information of the patient and cytomorphological features of the smears were studied and the samples were categorized into benign (with or without infection/ SBP), suspicious of malignancy or malignant.

Results: Our study showed that the mean age was 55.67 years. Out of 30 cases, 14 were males and 16 were females, with male to female ratio being 7:8. Out of 30 samples, 26 (86.66%) were benign, 2 (6.66%) were suspicious of malignancy, and 2 (6.66%) were positive for malignancy. The most frequent clinical diagnosis was Alcoholic Liver disease comprising of 8 (26.66%) cases. The predominant cell type was Lymphocyte in 14 (46.66%) of cases, Neutrophil in 9 (30%) of cases, Reactive

mesothelial cell in 5 (16.66%) of cases and atypical/malignant cells in 2 (6.66%) of cases. Histiocytic cells were also seen.

Conclusion: We concluded that non neoplastic and neoplastic conditions causing ascites can be diagnosed on ascitic fluid cytology. Increased neutrophil count is strongly suggestive of spontaneous bacterial peritonitis (SBP). The careful cytomorphological examination of ascitic fluid is a valuable, simple, rapid, inexpensive and reliable technique in the differential diagnosis of ascites, particularly in resource limited settings.

Keywords: Cytology, Ascites, Malignancy, Benign, Lymphocyte.

INTRODUCTION

Peritoneal fluid in peritoneal cavity keeps the surface moist and lubricated so that movement of adjacent or apposing membrane surfaces occurs with minimal friction. In diseased condition, greater amount of fluid accumulates in the cavity by a process of effusion. The patient with peritoneal effusion is said to have ascites and the fluid is called ascitic fluid.[1]

Ascites is one of the most common problems amongst the various clinical problems confronting a physician, and ascitic fluid analysis is the most effective way to diagnose it. Fluid accumulation in the abdomen was recognized in ancient times. Lucke and Kleb were apparently the first investigators who recognized the presence of malignant cells in ascitic fluid in 1867. In 1882, Quincke was credited for detailed description of ovarian and lung cancers in serous effusions. In recent years with the availability of several commercially available antibodies, diagnosis and typing of malignant cells in serous fluids has become more reliable, obviating the time consuming and expensive electron microscopic examination.[2]

Clinically, ascites is a consequence or complication of a number of diseases; including hepatic, cardiac and renal diseases, infections and malignancy.[3] 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.[4]

Cytological techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumours in effusions.[5]

Abdominal paracentesis is the single most important procedure and should be an early step in evaluating a patient with ascites. Paracentesis can be performed easily in minutes and is a minimally invasive procedure.

Early and accurate diagnosis often depends on appropriate ascitic fluid analysis. Paracentesis should be performed in all patients with new onset ascites. Patient with known liver disease with clinical deterioration also needs to have a paracentesis with a determination of ascitic fluid neutrophil and leucocyte count, and adequate bacteriological cultures.

Since the time reports on effusion cytology have started to appear in the medical literature, and serous effusion cytology now is a routine diagnostic procedure worldwide. In recent years, with the availability of several commercially available antibodies, diagnosis and typing of malignant cells in serous fluids has become more reliable, obviating the time-consuming and expensive electron microscopic examination of effusion cell blocks.[2]

The gross appearance of the fluid can provide useful diagnostic information. The clear straw coloured appearance of most ascitic fluids reflects the most common aetiology, namely cirrhosis. Samples which remain homogeneously blood stained throughout the tap could indicate malignancy, pancreatitis, TB, intestinal infarction or recent abdominal trauma. Blood-stained ascites is often thought to be a characteristic of peritoneal carcinomatosis, but an ascitic fluid RBC count of >10,000/mm^3 was only found in 8.3% of peritoneal carcinomatosis patients and 22% of malignancy-related ascites overall.[6] Peritoneal carcinomatosis accounts for two-thirds of patients with malignancy-related ascites and nearly all of these have positive cytology, due to the shedding of viable malignant cells from the peritoneal seedlings into the ascitic fluid.[7] By contrast, ascitic fluid cytology is positive in less than 10% of patients with hepatocellular carcinoma and is also usually negative in those with liver metastases.[6] With the exception of suspected ovarian malignancy, the emphasis placed on ascitic fluid cytology in the early assessment of ascites is felt to be misplaced since this has a sensitivity of only 60%, although 100% specificity.[8] The aim of this study to correlate the cytological features of the ascetic fluid with clinical data at newly established tertiary care center.

MATERIALS & METHODS

A prospective study was conducted in the Department of Pathology in collaboration with department of general medicine, in a tertiary health care centre over a period of one year. The study included 30 patients who presented with ascites in the Department of General medicine. Relevant clinical information regarding age, sex and symptoms was obtained. Samples received were processed immediately. Fresh fluid was submitted for physical examination noting characteristics like volume, appearance, and colour. Cytological examination was performed to reveal important information regarding the causes of ascites and classified as benign (with or without infection/ SBP), suspicious or malignant conditions.

INCLUSION CRITERIA

All ascitic fluid samples sent to cytology section, Department of pathology, GMC Barmer, Rajasthan.

EXCLUSION CRITERIA

Unlabeled specimens, samples less than 2 ml in volume were excluded from the study.

METHODS

The ascitic tap samples from hospitals were analysed. Samples were checked for adequacy, any leakage of container or contamination, a unique number was assigned to the sample, clinical details were checked from the case sheet and were entered in the register.

PROCESS

Majority of the samples were processed immediately. But in some cases, if there was delay, samples were stored in refrigerator at 4^oC. 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.
Cytomorphology of the cells was studied and documented paying attention to cellular arrangement, cytoplasmic features and nuclear characteristics.

INTERPRETATION OF DATA

Clinical information of the patient and cytomorphological features of the smears were studied and the samples were categorized into benign (with or without infection/ SBP), suspicious of malignancy or malignant.

RESULTS

In this study, the age distribution ranged from 15 years to 85 years, with mean age being 55.67 years. Maximum cases ranged between 40 to 49 years (30%). Out of 30 cases, 14 were males and 16 were females, with male to female ratio being 7:8. Out of 30 samples, 26 (86.66%) were benign, 2 (6.66%) were suspicious of malignancy, and 2 (6.66%) were positive for malignancy (table 1).

Table 1: Age distribution of cytological diagnosis

Age Group	Benign	Suspicious	Malignant	Total
20-29	2 (6.66%)	0 (0%)	0 (0%)	2 (6.66%)
30-39	4 (13.33%)	0 (0%)	0 (0%)	4 (13.33%)
40-49	7 (23.33%)	1 (3.33%)	1 (3.33%)	9 (30%)
50-59	7 (23.33%)	0 (0%)	0 (0%)	7 (23.33%)
60-69	3 (10%)	1 (3.33%)	1 (3.33%)	5 (16.66%)
70-79	2 (6.66%)	0 (0%)	0 (0%)	2 (6.66%)
80-89	1 (3.33%)	0 (0%)	0 (0%)	1 (3.33%)
TOTAL	26 (86.66%)	2 (6.66%)	2 (6.66%)	30 (100%)

The most frequent clinical diagnosis was Alcoholic Liver disease comprising of 8 (26.66%) cases which was followed by Congestive heart failure/severe anemia cases which comprised 5 (16.66%) of cases. 3 (10%) cases were of Chronic liver disease other than alcoholic liver disease. 2 (6.66%) cases were suspicious of malignancy while 2 (6.66%) cases were known cases of malignancy (table 2).

Table 2: Distribution of clinical diagnosis

Clinical Diagnosis	Number	Percentage
Alcoholic liver disease	8	26.66%
Chronic liver disease (other than ALD)	3	10%
Congestive heart failure/severe anemia	5	16.66%
Suspected malignancy	2	6.66%
Known case of malignancy	2	6.66%
Post laparotomy cases	2	6.66%
Chronic kidney disease/renal failure	2	6.66%
Ovarian tumour/cyst	2	6.66%
Abdominal tuberculosis	1	3.33%
Acute kidney injury	1	3.33%
Cholelithiasis with ascites	1	3.33%
Septicemia	1	3.33%
Total	30	100

The physical characteristics of the fluid sample received were noted, including volume, colour, and appearance. Majority of the fluids were straw coloured (40%) (table 3).

Table 3: Physical characteristics

Clinical Diagnosis	Straw	Yellowish	Turbid	Haemorrhagic
Alcoholic liver disease (26.66%)	2 (6.66%)	3 (10%)	2 (6.66%)	1 (3.33%)
Chronic liver disease (other than ALD) (10%)	1 (3.33%)	2 (6.66%)	0	0
Congestive heart failure/severe anemia (16.66%)	4 (13.33%)	0	1 (3.33%)	0
Suspected malignancy (6.66%)	1 (3.33%)	0	1 (3.33%)	0
Known case of malignancy (6.66%)	0	0	1 (3.33%)	1 (3.33%)
Post laparotomy cases (6.66%)	0	0	2 (6.66%)	0
Chronic kidney disease/renal failure (6.6%)	1 (3.33%)	0	1 (3.33%)	0
Ovarian tumour/cyst (6.66%)	1 (3.33%)	0	1 (3.33%)	0
Abdominal tuberculosis (3.33%)	1 (3.33%)	0	0	0

Acute kidney injury (3.33%)	0	0	1 (3.33%)	0
Cholelithiasis with ascites (3.33%)	0	0	1 (3.33%)	0
Septicaemia (3.33%)	1 (3.33%)	0	0	0

The predominant cell type was Lymphocyte in 14 (46.66%) of cases, Neutrophil in 9 (30%) of cases, Reactive mesothelial cell in 5 (16.66%) of cases and atypical/malignant cells in 2 (6.66%) of cases. Histiocytic cells were also seen (table 4).

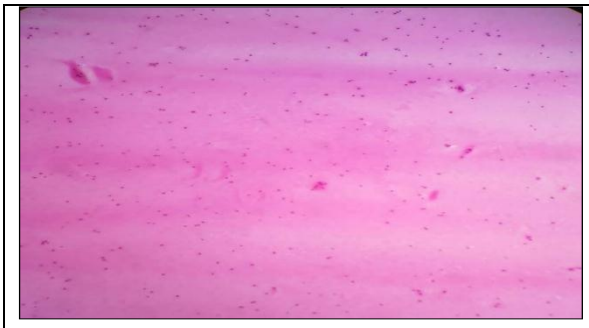
Table 4: Distribution of cytological diagnosis

Predominant Cell Type	Number Of Cases	Percentage Of Cases
Lymphocytes	14	46.66%
Neutrophils	9	30%
Reactive Mesothelial cells	5	16.66%
Atypical cells	2	6.66%

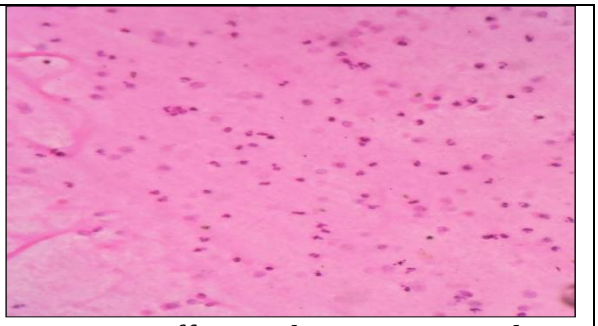
Smears showing predominantly scant lymphocytes along or reactive mesothelial cells and a few other inflammatory cells were categorized as benign. The large amount of polymorphous population of lymphocytes as the predominant cell pattern were considered benign with suspicion of tuberculosis. The predominantly neutrophils with absolute neutrophil count exceeding 250/cumm were categorized as benign with SBP/infectious.

Smears which showed pleomorphic cells, with atypical nuclear characteristics, sometimes arranged in characteristic acinar pattern, were considered as suspicious of malignancy or malignant. The minimum cell count obtained was 40/cumm in a patient of ascites with alcoholic liver disease, while the maximum cell count obtained was 8500/cumm in a patient with ascites and renal failure.

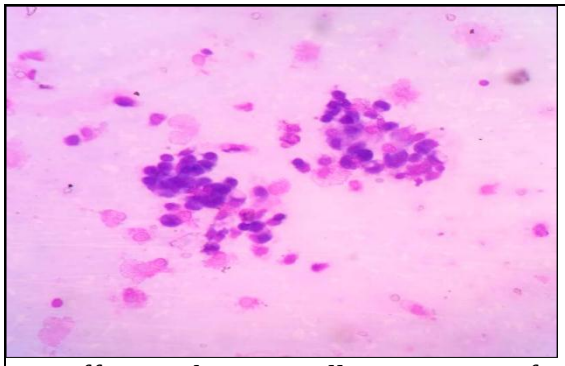
Figure 1: Cytomorphology of different lesions



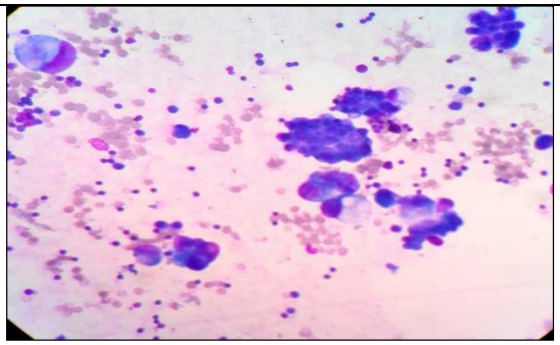
Benign effusion showing predominantly scattered lymphocytes



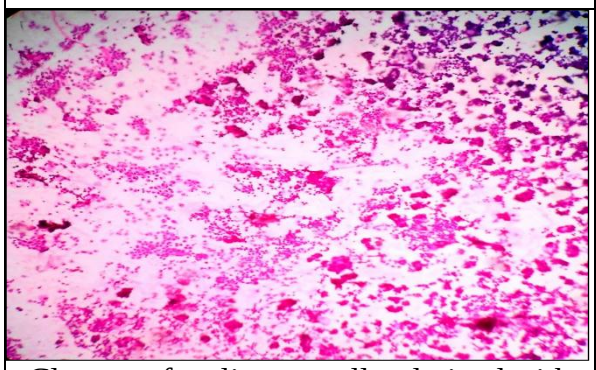
Benign effusion showing scattered neutrophils, lymphocytes and mesothelial cells



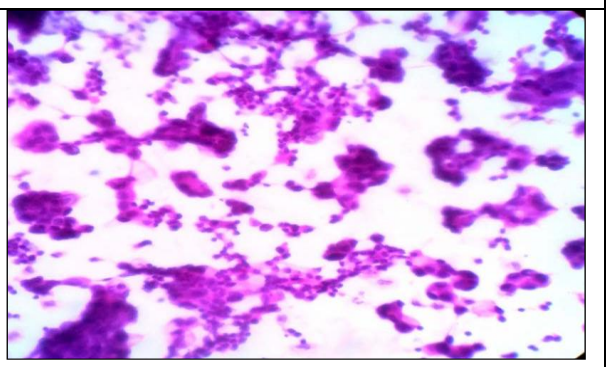
Effusion showing cells suspicious of malignancy



Malignant cells in acinar pattern-Malignant Effusion, 40X, Geisma stain



Clusters of malignant cells admixed with inflammatory cells-Effusionpositive for malignancy, 10X, H&E.



Dense population of malignant cells arranged in acinar patter,clusters and nests-Effusion positive for malignancy. 40 X, H&E.

DISCUSSION

Ascites means presence of excess fluid in the peritoneal cavity, normally upto 50 ml of fluid is present. Ascites is a common clinical condition that poses a diagnostic problem to the clinicians.[9]

There has been a worldwide surge of interest in the cytological detection of cancer cells since Quincke published the results of his histological and chemical studies on chylous ascites. Reservations held in earlier years about the usefulness of searching for cancer cells in serous effusions have been largely overcome by several published results confirming the usefulness of the method.[10]

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.[11] The most useful test in establishing the differential diagnosis of ascites is ascitic fluid cytology and ascitic fluid cell count.

Aspiration of serous cavities is a simple and relatively noninvasive techniqueto achieve a diagnosis. Cytological examination of serous fluid is of paramount importance, it reveals information about inflammatory conditions of serous membrane, infection by bacteria, fungi, virus, finding of cancer cells and also supplyevidence of fistulous connection with a serous cavity. Cytological examination not only helps for the diagnosis of cancer but

also for staging and prognosis of disease. It is a complete diagnostic modality which aims at pointing out the etiology of effusionas well as prognosis of disease.[12]

The literature states that the initial management of the patient presenting with ascites should be a thorough history with examination, and this should then direct any further laboratory assessment, with cell count and differential, albumin, and culture being mandatory. In addition, serum albumin should be obtained to calculate the SAAG (serum ascites albumin gradient) as an important determinate of the differential count. Cytology is to be performed only when there is a suspicion of malignancy. Runyon suggested the following three criteria as indications to proceed with cytology: (i) a history of cancer, (ii) no physical findings suggestive of liver disease, and (iii) an initial ascitic fluid sample with a high lymphocyte count (500 cells/mm^3) and few neutrophils.[13]

In the present study, 86.66% cases were benign, 6.66% were suspicious of malignancy and 6.66% were positive for malignancy. These results correlated with studyconducted by Dowerah E et al[12] in which 85% cases were benign, 8% suspicious and 6% were positive for malignancy. Also, similar results were found in a study by Kumawat et al[14] in which 92% cases were classified as non-neoplastic and 8% as neoplastic.

In this study, the age distribution ranged from 15 years to 85 years, with meanage being 55.67 years. Maximum cases ranged between 40 to 49 years (30%), this is comparable with study conducted by Khan FY in 2001 in which mean age was 52.9 years with standard deviation of 14.8.[15] Another study done by Shikha NG in 2014 showed that maximum patients (36%) fell in the age range of 40-49 years.[9]

Our study correlated with the study by Karoo et al[13] which showed female preponderance, while the study by Shikha NG[9] revealed male preponderance of ascites. This may be due to variation in lifestyle habits and alcohol consumption behaviour in different areas.

40% of the fluids received were straw coloured. Majority of cases that were later recognized as to be with infection/SBP were turbid in appearance. Thus, it can be concluded that an approximate evaluation of the presence or absence of infection can be made by the gross turbid appearance of the fluid received.

Hepatic pathology related cases (alcoholic and nonalcoholic liver disease) comprised of 40% of cases, out of which 28% were benign with lymphocyte or mesothelial cells as predominant cell population, while the remaining 12% had neutrophils as predominant cell population and were diagnosed as with secondary infection/SBP. We concluded that SBP is a very common occurrence in ascites patients and therefore cytological examination of these fluids is of paramount importance as SBP can be potentially fatal if not treated in time.[16]

Predominant cell population seen was lymphocyte in 46.66% of cases. This is comparable with the study conducted by Junaid et al where lymphocyte was the predominant cell population in 40% of cases.[10]

Majority of fluids had scant to moderate cellularity with predominantly lymphocytes and few reactive mesothelial cells. Large number of lymphocytes point towards a diagnosis of tuberculosis or may be due to presence of a lymphoma.

Neutrophil count >250/cumm is taken as SBP/ infected ascites. Recognizing SBP is important as it may have a grave prognosis for the patient unless early treatment is

instituted. Generally, these samples were turbid in appearance and had neutrophils in clusters and sheets admixed with reactive mesothelial cells and degenerating cells.
Presence of an overwhelming number of neutrophils masking the other cell population and the reactive nuclear changes in the other cells due to presence of inflammation may either mask the atypical cells or may lead to suspicious diagnosisin a benign condition.
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.
The differentiation between malignancy-related and non-malignant ascites isimportant for further diagnostic and therapeutic procedures. Cytologic investigation of ascitic fluid is specific but may produce a large percentage of false-negative results; its sensitivity ranges between 40% and 70%.[18]

CONCLUSION

We concluded that non neoplastic and neoplastic conditions causing ascites can be diagnosed on ascitic fluid cytology. Increased neutrophil count is strongly suggestive of SBP. The careful cytomorphological examination of ascitic fluid is a valuable, simple, rapid, inexpensive and reliable technique in the differential diagnosis of ascites, particularly in resource limited settings.

REFERENCES

1. Walter JB, Talbot JC. Hepatic failure and jaundice. In: Walter and Israel General Pathology. 7th ed. Churchill Livingstone; 1998:242-5.
2. Nguyen G. Essentials of fluid cytology. Surrey, BC: Gia-Khanh Nguyen; 2010 :9-12.
3. Huang LL, Xia HH, Zhu SL. Ascetic fluid analysis in the differential diagnosis of ascitis. Focus on cirrhotic ascites. JCTH.2014;2: 58-64.
4. Anita B, & Ahuja JM. Evaluation of coelomic fluids and its clinical correlation with cytologic diagnosis. J Cytol Histol.2016;7(2): 1-19.
5. Koss L, Melamed M, Koss L. Koss' diagnostic cytology and its histopathologic bases. Philadelphia: Lippincott Williams & Wilkins; 2006 :920-1020.
6. Runyon, BA, Hoefs, JC, Morgan, TR. Ascitic fluid analysis in malignancy-related ascites. Hepatology 1988;8:1104–9.
7. Cardozo, PL. A critical evaluation of 3000 cytologic analyses of pleural fluid, ascitic fluid and pericardial fluid. Acta Cytol 1966;10:455–60.
8. Karoo, RO, Lloyd, TD, Garcea, G, Redway, HD, Robertson, GS. How valuable is ascitic cytology in the detection and management of malignancy? Postgrad Med J 2003;79:292–4.
9. Shikha NG. A Cytological Study of Peritoneal Fluids and Its Clinicobiochemical Correlation. IOSR Journal of Dental and Medical Sciences. 2014 Feb;13(2):12-5.

10. Junaid TA. Cytologic diagnosis of ascitic fluid in Ibadan, Nigeria. Journal of the National Medical Association 1980;72(7):669-72.
11. Cibas ES. Pleural, pericardial and peritoneal fluids. In: Cytology: Diagnostic principles and clinical correlate. Cibas ES and Ducatman BS editors.3rd ed. Philadelphia: Elsevier:129-153,2009.
12. Dowerah E, Das S. Cytological evaluation of peritoneal fluid with special reference to malignancy. International Journal of Biomedical Research 2014;5:6.
13. Karoo ROS, Lloyd TDR, Garcea G, Redway HD, Robertson GSR. How valuable is ascitic cytology in the detection and management of malignancy? Postgrad Med J 2003;79:292-4.
14. Kumavat PV, Rathod UD, Kulkarni M, Sulhyan KR. Cytological Study of Malignant Neoplasms in Pleural, Peritoneal and Pericardial Effusions. Bombay Hospital Journal 2015;57(3):267-72.
15. Khan FY. Ascites in the state of Qatar: aetiology and diagnostic value of ascetic fluid analysis. Singapore Med J 2007 May;48(5):434-9.
16. Bankar S, De A, Baveja S. Study of ascitic fluid for diagnosis of spontaneous bacterial peritonitis (sbp) in adult patients with cirrhosis. International Journal of Medical and Applied Sciences. 2014;3(1):1-9.
17. Patel NP, Taylor CA, Levine EA, Trupiano JK, Giesinger KR. Cytomorphologic features of primary peritoneal mesothelioma in effusion, washing and fine needle aspiration biopsy specimens. Am J Clin Pathol.2007;128: 414-22.
18. Gerbes AL, Jiingst D, Xie Y, Permanetter W, Paumgartner G. Ascitic Fluid Analysis for the Differentiation of Malignancy-Related and Nonmalignant Ascites-Proposal of a Diagnostic Sequence. Cancer 1991;68:1808-14.