Original Article ISSN (O):2395-2822; ISSN (P):2395-2814

Study of Ascitic Fluid by Liquid Based Cytology and Its Comparision with Conventional Cytosmears and Cell Block Preprations.

Rishu Mittal[1], Kundal Ramesh[2], Singh harpal[3], Deepika Gupta[1], Bhavleen Kaur[1], H.S.Sandhu[4]

[1]Junior Resident, Department of Pathology, Govt. Medical College Patiala.
[2]Professor, Department of Pathology, Govt. Medical College Patiala.
[3]Associate Professor, Department of Pathology, Govt. Medical College Patiala.
[4]Professor, Department of Medicine, Govt. Medical College Patiala.

Received: September 2016
Accepted: September 2016

ABSTRACT

Background: Ascites refers to increased volume of fluid collecting within peritoneal cavity which becomes clinically detectable when atleast 500 ml has accumulated. Cytological examination of ascitic fluid gives information about inflammatory and noninflammatory lesions including malignancies, which is done by conventional cytosmears, SurePath liquid based cytological smears and cell block preparations. **Aims:** The aim of our study was to study the different causes of ascites and their comparison on liquid based cytology with conventional cytology and cell blocks. **Methods:** Ascitic fluid was obtained from 75 patients of either sex. Microscopic examined was carried out by SurePath liquid based cytology, conventional fixed sediment smears, and cell blocks. **Results:** Observations were categorised into inflammatory, malignant and inconclusive. Out of 75 cases examined by conventional smears, cytological diagnosis of inflammatory or benign was rendered in 45(60%), 7(9.3%) were diagnosed as malignant and 5(6.7%) were given suspicious of malignancy and 18(24%) were inconclusive. By liquid based cytology 53(70.7%) were rendered inflammatory or benign, 12 (16%) as malignant, 2(2.7%) as suspicious of malignancy and 8(10.7%) were rendered inconclusive. By cell block methodology 52(69.3%) were rendered inflammatory or benign, 11(14.7%) as malignant and 12(16%) as inconclusive. Statistical analysis: Revealed that liquid based cytology was most sensitive (85.71%) and accurate (97.33%) method for analysis of ascitic fluid and conventional smears were least sensitive (50%) and accurate (90.67%). **Conclusion:** Liquid based cytology showed more sensitivity and accuracy than conventional cytosmears and cell block methods in diagnosing malignant lesions.

Keywords: Ascitic fluid, liquid based cytology, conventional cytology, cell blocks.

INTRODUCTION

Peritoneum encloses gastrointestinal organs, and consists of mesothelium composed of a single layer of flat cells, supported by connective tissue and an appropriate vascular and nervous apparatus. The parietal and visceral layers are separated by a cavity filled with lubricating fluid that facilitates the movements of the two layers against each other. Normal amount of peritoneal fluid is 50 ml.[1]

Name & Address of Corresponding Author
Dr. Harpal Singh
Associate Professor,
Department of Pathology,
Govt. Medical College, Patiala.

Ascites refers to the collection of excess fluid in the peritoneal cavity. It usually becomes clinically detectable when atleast 500ml has accumulated, but many liters may collect and cause massive abdominal distention. Ascitic fluid is usually a serous fluid having less than 3gm/dl of protein (largely albumin) as well as the same concentrations of solutes such as glucose, sodium and potassium as in the blood. The fluid may contain a scant number of mesothelial cells and mononuclear leucocytes. Influx of neutrophils suggests secondary infection, whereas red cells point to possible disseminated intra-abdominal cancer.[2]

The ascitic fluid is classified as transudate or exudate. The transudate is clear, straw-coloured fluid characterized by a low specific gravity, and low protein content. The cellular components of transudate are scanty and are limited to a few mesothelial cells and leukocytes. The exudates on the other hand is cloudy or opaque fluid of various colors and characterized by high protein content and a high specific gravity. The exudate is rich in fibrin and may coagulate on standing and usually contain a significant population of cells that are the target of cytologic investigations. Most common cause of ascites is portal hypertension related to cirrhosis.[1,3]

Conditions that can cause ascites include:-

1. Increased hydrostatic pressure associated with portal hypertension: cirrhosis, alcoholic hepatitis, fulminant hepatic failure, fatty liver of pregnancy, hepatic fibrosis etc.
2. Decreased colloid osmotic pressure secondary to hypoalbuminaemia: nephrotic syndrome, malnutrition.
3. Increased permeability of peritoneal capillaries: bacterial peritonitis.
4. Leakage of fluid into the peritoneal cavity: bile ascites.
5. Malignant conditions: hepatocellular carcinoma etc. [4]

The confirmation or exclusion of intra-abdominal malignancy is almost entirely the reason for cytologic examination of ascitic fluid. Cytology is also useful for predicting the prognosis of gynaecological, gastric, pancreatic and colorectal malignancies. Finding of malignant cells in ascitic fluid usually denotes advanced disease and cautions against a major surgical assault or excludes surgery entirely. However, fluid cytology shows tumour cells only when tomour cells are lining the peritoneum, not when peritoneum is not involved.[5,6]

ThinPrep was approved for cervico-vaginal (Pap test) cytology in 1996 and SurePath in 1999 and both have since also been used for non-gynaecological cytology. In the liquid based prepration (LBP), instead of being smeared, cells are rinsed into a liquid preservative collection medium and processed on automated devices.[7]

In recent years liquid-based cytology has emerged as an alternative to conventional cytopreparatory methods. Many laboratories have successfully applied this technique to body fluids, brushing samples, and fine-needle aspiration. Most comparative studies have shown the liquid based cytology to perform as well as or better than conventional preparations in non-gynecologic cytology.[8]

Liquid-based cytology (LBC), enables cells to be suspended in a monolayer. It makes better cytological assessment possible with improved sensitivity and specificity, since fixation is better and nuclear details are well preserved in the technique. [9]

The cytological examination of fluids by means of smears, however carefully prepared, leaves behind a large residue that is not further investigated but that might contain valuable diagnostic material. This residual material can be evaluated in a simple and expedient fashion by treating it as a cell block, embedded in paraffin, and examined in addition to the routine smears. [10]

Beale (1895) [11] introduced paraffin block method for serous effusions. In 1896 Bahrenbug first described the cell block technique and it was commonly used after Mandlebaum reported finding of actinomyces in a cell block.

The cytologic appearances of the cells in the liquid-based medium are different and staff of laboratories adopting the new system have to be specially trained. Laboratories offering direct-to-vial testing may be able to overcome the difficulties with some cases by performing cell block sections of residual materials in the samples.[12]

MATERIALS AND METHODS

The study comprises ascitic fluid examination from 75 patients which were received in the department of Pathology, Government Medical College, Patiala. Ascitic fluid specimens received in the laboratory were subjected to both gross and microscopic examination.

The features observed on gross examination of ascitic fluid were volume of the ascitic fluid specimen, colour of the ascitic fluid specimen and any other special character (such as turbidity, floating tissue fragments)

The microscopic examination of ascitic fluids was carried out by three methods SurePath Liquid based cytology, conventional fixed sediment smears and cell blocks. The ascitic fluid was processed. First conventional fixed sediment smears were prepared and stained with Papanicolaou staining and May-Grunwald-Giemsa staining. Secondly, cell blocks were prepared by fixed sediment method and sections were stained with Haematoxylin and Eosin. Lastly, SurePath Liquid based cytology smears were prepared and stained with BD PrepStain™ . Liquid based cytology, conventional, cell blocks, smears were thoroughly examined under the microscope for the various types of cells. The observations were categorized into inflammatory, malignant, inconclusive and sucpicious.

RESULTS

Out of 75 samples of ascitic fluid received, 28 (37.3%) patients of alcoholic liver disease/ cirrhosis/ chronic liver disease and 19 (25.3%) had adnexal mass and 9 (12%) patients have infective pathology, 3 (4%) patients have liver metastasis or primary in the liver, 3 (4%) patients have other malignancies and 13 (17.3%) patients were those who have unknown cause for ascitis [Table 1].

Other parameters of ascitic fluid study shown in tables no. 2-13 below and microscopic examination of the ascitic fluid study shown through figures 1-8.

Table 1: Distribution of cases according to clinical diagnosis.

Clinical Diagnosis	Number of Patients	Percentage
Liver disease and Cirrhosis	28	37.3%
Adnexal mass	19	25.3%
Infective	9	12%
Liver metastasis and	3	4%

primary		
Other malignancies	3	4%
Unknown cause	13	17.3%
Total	75	100%

Table 2: Distribution of cases according to colour of ascitic fluid.

Colour of Ascitic Fluid	Number of Patients	Percentage
Red	25	33.3%
Yellow	22	29.3%
Straw	21	28%
Brown	4	5.3%
Clear	2	2.7%
White	1	1.3%
Total	75	100%

Table 3: Distribution of cases on the basis of cytological analysis by conventional smear.

Diagnosis	Number of Patients	Percentage
Inconclusive	18	24%
Inflammatory/Benign	45	60%
Suspicious	5	6.7%
Malignant	7	9.3%
Total	75	100%

Table 4: Distribution of cases on the basis of cytological analysis by liquid based cytology.

Diagnosis	Number of Patients	Percentage
Inconclusive	8	10.7%
Inflammatory/Benign	53	70.7%
Suspicious	2	2.7%
Malignant	12	16%
Total	75	100%

Table 5: Distribution of cases on the basis of cytological analysis by cell block method.

Diagnosis	Number of Patients	Percentage
Inconclusive	12	16%
Inflammatory/benign	52	69.3%
Malignant	11	14.7%
Total	75	100%

Table 6: Distribution of cases according to final diagnosis on the basis of benign or malignant.

Diagnosis	Number of Patients	Percentage
Benign	61	81.3%
Malignant	14	18.7%
Total	75	100%

Table-7: Distribution of cases according to cause of ascitic fluid.

Causes	Number of Patients	Percentage
Inflammatory (Acute and Chronic)	39	52%
Cirrhotic or Chronic liver disease	16	21.3%
Adenocarcinoma Ovary	6	8%
Adenocarcinoma GIT	1	1.3%
Adenocarcinoma endometrium	1	1.3%
Carcinoma Breast	1	1.3%
Hepatocellular carcinoma	1	1.3%
Other Malignancies	4	5.3%
Inconclusive	6	8%
Total	75	100%

Table 8: Correlation of cytological diagnosis by conventional smear with final diagnosis.

Conventional Smear	Final diagnosis		Total
	Benign (%age)	Malignant (%age)	
Inconclusive	17 (94.4%)	1 (5.6%)	18 (100%)
Inflammatory/ benign	43 (95.6%)	2 (4.4%)	45 (100%)
Suspicious	1 (20%)	4 (80%)	5 (100%)
Malignant	0 (0%)	7 (100%)	7 (100%)
Total	61 (81.3%)	14 (18.7%)	75 (100%)

Table 9: Correlation of cytological diagnosis by liquid based cytology with final diagnosis.

LBC	Final diagnosis		Total
	Benign	Malignant	
Inconclusive	8 (100%)	0 (0%)	8 (100%)
Inflammatory/Benign	52(98.1%)	1 (1.9%)	53 (100%)
Suspicious	1 (50%)	1 (50%)	2 (100%)
Malignant	0 (0%)	12 (100%)	12 (100%)
Total	61 (81.3%)	14 (18.7%)	75 (100%)

TABLE 10: Correlation of histological diagnosis by cell block with final diagnosis.

Cell Block	Final diagnosis		Total
	Benign (%age)	Malignant (%age)	
Inconclusive	11 (91.67%)	1 (8.33%)	12 (100%)
Inflammatory/benign	50 (96.2%)	2 (3.8%)	52(100%)
Malignant	0 (0%)	11 (100%)	11 (100%)
Total	61 (81.3%)	14 (18.7%)	75 (100%)

Twelve cases which were inconclusive by Cell Block, out of which 6 were inconclusive, 5 were inflammatory/ benign and 1 was given malignant by liquid based cytology. Out of 52 cases which were diagnosed inflammatory or benign by cell block, 47 cases were diagnosed inflammatory/ benign, 2 were malignant, 1 was suspicious and 2 were diagnosed inconclusive by liquid based cytology. Out of 75 cases, 9 cases were given malignant by both liquid based cytology and cell block.

In the present study, out of 75 cases, 12 cases were diagnosed malignant by liquid based cytology and 11 cases were diagnosed malignant by cell block.

More cases were inconclusive by cell block than liquid based cytology [Table 11].

Liquid based cytology was slightly superior to cell block.

Table 11: Comparison of diagnosis of liquid based cytology with cell block.

Cell block	Liquid based cytology				Total
	Inconclusive	Inflammatory/Benign	Suspicious	Malignant	
Inconclusive	6 (50%)	5 (41.7%)	0 (0%)	1 (8.3%)	12 100%)
Inflammatory/ Benign	2 (3.8%)	47 (90.4%)	1 (1.9%)	2 (3.8%)	52(100%)
Malignant	0 (0%)	1 (9.1%)	1 (9.1%)	9 (81.8%)	11(100%)
Total	8 (10.7%)	53 (70.7%)	2 (2.7%)	12 (16%)	75 100%)

Out of 75 cases, 12 cases were diagnosed malignant by liquid based cytology out of which 7 were diagnosed malignant, 3 were suspicious, 1 was inflammatory and 1 was inconclusive by conventional smears.

Out of 18 cases were given inconclusive by conventional smears, 8 were given inconclusive, 9 were inflammatory/ benign and 1 was given malignant by liquid based cytology.

Three suspicious, 1 inflammatory and 1 inconclusive by conventional smears were given malignant by liquid based cytology.

This study showed liquid based cytology was better than conventional smears [Table 12].

Table 12: Comparison of liquid based cytology with conventional smear.

Conventional smear	LBC				Total
	Inconclusive	Inflammatory/ Benign	Suspicious	Malignant	
Inconclusive	8 (44.4%)	9 (50%)	0 (0%)	1 (5.6%)	18 100%)
Inflammatory/ Benign	0 (0%)	44 (97.8%)	0 (0%)	1 (2.2%)	45(100%)
Suspicious	0 (0%)	0 (0%)	2 (40%)	3 (60%)	5 100%)
Malignant	0 (0%)	0 (0%)	0 (0%)	7 (100%)	7(100%)
Total	8 (10.7%)	53 (70.7%)	2 (2.7%)	12 (16%)	75(100%)

11 cases were given malignant by cell block out of which 6 were given malignant, 3 were suspicious and 2 were given benign by conventional smear.

One case which was inconclusive by cell block was given malignant by conventional smears [Table 13].

This study showed cell block was better than conventional smears.

Table 13: Comparison of cell block with conventional smear.

Variables	Cell Block			Total
	Inconclusive	Inflammatory/Benign	Malignant	
Inconclusive	9 (50%)	9 (50%)	0 (0%)	18 (100%)
Inflammatory/Benign	2 (4.4%)	41 (91.1%)	2 (4.4%)	45 (100%)
Suspicious	0 (0%)	2 (40%)	3 (60%)	5 (100%)
Malignant	1 (14.3%)	0 (0%)	6 (85.7%)	7 (100%)
Total	12 (16%)	52 (69.3%)	11 (14.7%)	75 (100%)

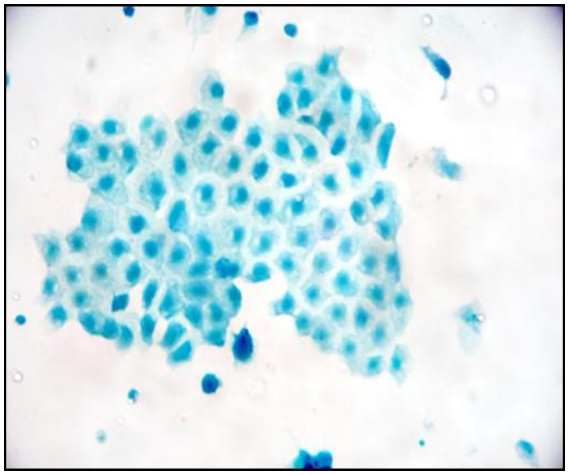

Figure 1: Photomicrograph showing sheet of reactive mesothelial cells with clear window in between the cells (LBCx400)

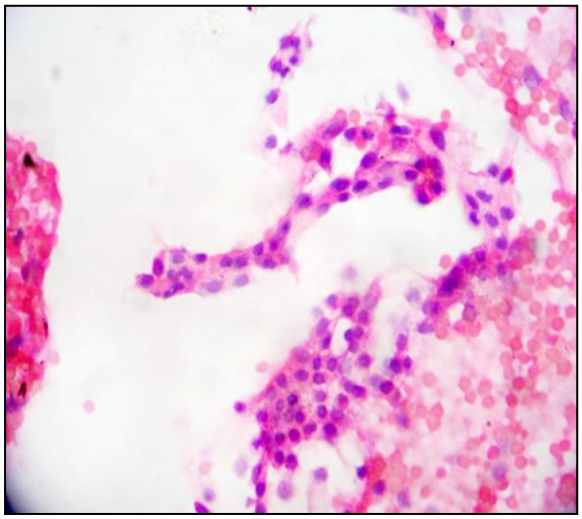

Figure 2: Photomicrograph of cell block showing sheet of mesothelial cells in the background of RBC's (H and E Stain x 400)

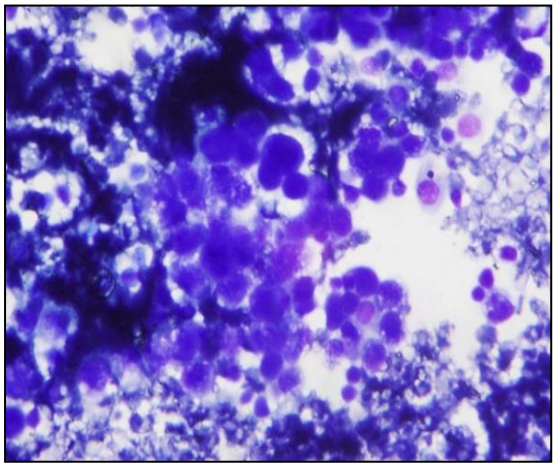

Figure 3: Photomicrograph showing malignant epithelial cells forming acini and clusters (MGGx400)

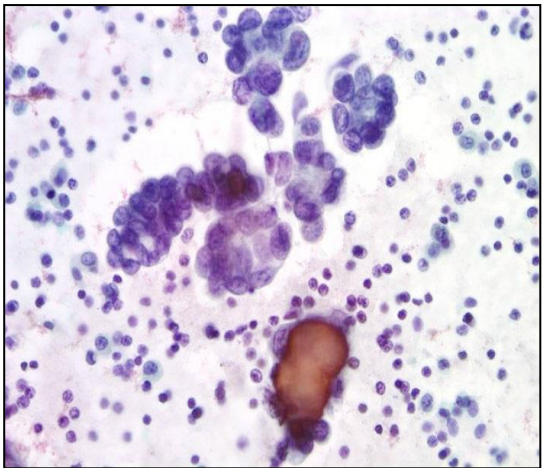

Figure 4: Photomicrograph showing malignant epithelial cells forming acini (LBCx400)

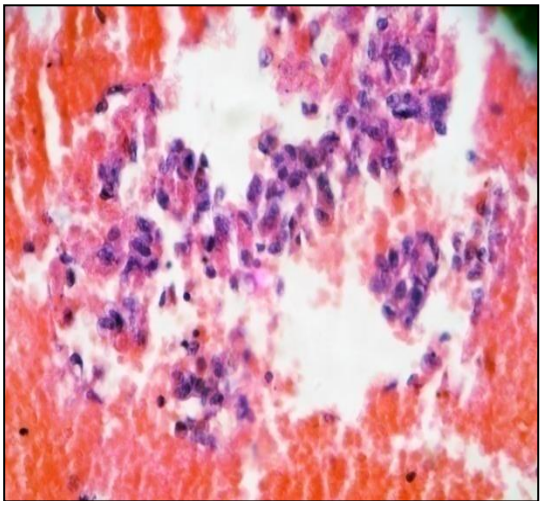

Figure 5: Photomicrograph of cell block showing clusters and acini of malignant epithelial cells (H and E stainx400)

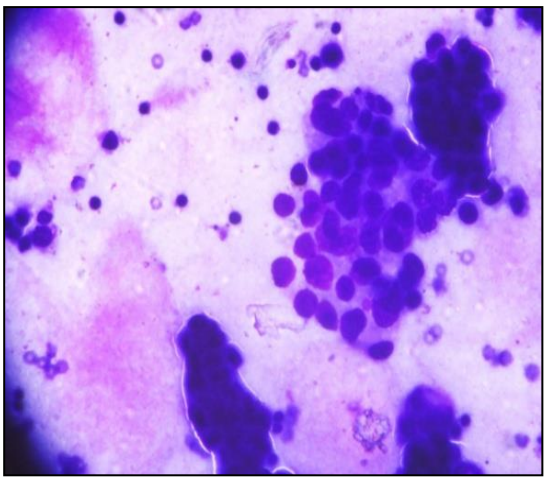

Figure 6: Photomicrograph showing malignant epithelial cells forming papillae (MGGx400)

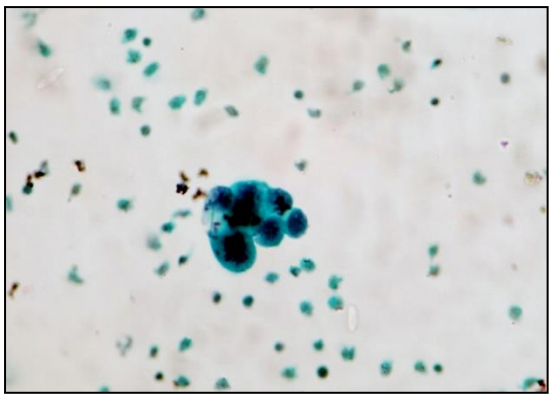

Figure 7: Photomicrograph showing cluster of malignant epithelial cells with abnormal mitosis (LBCx400)

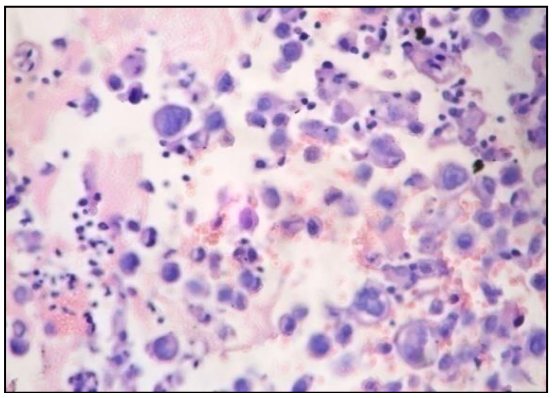

Figure 8: photomicrograph of cell block showing malignant epithelial cells: Cystadenocarcinoma ovary (H and E stain x400)

DISCUSSION

Out of 75 cases, 81.3% patients were benign and 18.7% patients were malignant. In the present study, out of 75 cases, benign effusions were seen in 81.3% of cases which were comparable with the results of studies done by Gandhi et al[13] in which benign cases were 82.14%, Karoo et al[14] in which benign cases were 83%, Pradhan et al[15] in which benign cases were 81.2%, Nathan et al[16] in which benign cases were 82.5% and Santwani and Vachhani[17] in which benign cases were 75.4%.

In the present study, out of 75 cases, 18.7% cases were diagnosed as malignant which were comparable with the results of studies done by Gandhi et al[13] in which malignant cases were 17.85%, Karoo et al[14] in which malignant cases were 17%, Pradhan et al[15] in which malignant cases were 18.8%, Nathan et al[16] in which malignant cases were 17.5%, and Santwani and Vachhani[17] in which malignant cases were 24.6%.

In the present study most common malignancy was adenocarcinoma and most common site of primary was ovary which were comparable with the results of Udasmith et al[11], Santwani and Vachhani[17], Karoo et al[14], Kumavat et al[18], Monte et al[19].

In the present study additional yield of malignancy by cell block was 5.3% which was less than the additional yield of studies done by thapar et al[10], Udasmith et al[11] (2012) and Santwani and Vachhani[17] in additional yield of malignancy by cell block was 13%, 13.63% and 10%, respectively.

Reason for the low yield of malignancy in this study may be due to technical errors such as inadequate sampling (less than 5 ml of ascitic fluid sent to the laboratory) or degenerated samples.

In the present study, benign cases diagnosed by liquid based cytology were 61 (81.3%), which were comparable with the results of Gabriel et al[20] in which benign cases were 76% but more than the study done by Qing[21] in which they were 60%.

This study showed liquid based cytology diagnosed suspicious and malignant were 2 (2.6%) and 12 (16%) respectively. The results of study were comparable with the results of study done by Gabriel et al[20] in which suspicious cases are 7 (2.4%) and malignant cases were 64 (21.9%). There was variation in the results of previous studies. Our results match with the results of Gabriel et al[20].

As compared to conventional smears, the interpretation of LBC smears were easy because cells were well preserved, concentrated in smaller area with a clean background and reduced number of erythrocytes did not obscure the diagnostic cells.

In the present study additional yield of malignancy by LBC was 6.7% which was less than the studies done by Gabriel et al[20] (2004) and Qing[21] (2007) in which additional yield was 12%.

This difference might be due to that LBC was not standardized yet for nongynaecologic samples. The results may be improved with time

CONCLUSION

It was thus concluded from the present study that Liquid based cytology showed more sensitivity and accuracy than conventional cytosmears and cell block methods in diagnosing malignant lesions. Cell blocks were more sensitive and accurate than conventional smears and less sensitive and accurate than liquid based cytology. Liquid based cytology and cell block yielded more cellularity with better architectural preservation than conventional cytosmears.

REFERENCES

1. Koss LG and Melamed MR. Diagnostic cytology and its histopathological bases; 5thedition; Lippincott Williams & Wilkins; Philadelphia:2006:920-2.
2. Kumar V, Abbas AL, Fausto N, Aster JC. Robbins and cotran pathological basis of disease. 7th ed. India: Elsevier; 2005:884.
3. Fauci AS, Braunwald E, Kasper DL, Hausar SL, Longo DL, Jameson JL et al. Harrison's Principles of internal medicine. 17thed; McGraw Hill; New York:2008;2:1978-9.

4. Balfe A, Barry S, Blake O, Cannon D, Healy M, Kilbane M et al. The Biochemistry of Body Fluids. Association of Clinical Biochemists in Ireland. 1st edition;2009:17-9.
5. Jha R, Shrestha HG, Sayami G, Pradhan SB. Study of effusion cytology in patients with simultaneous malignancy and ascites. Kathmandu Univ Med J (KUMJ). 2006;4(4):483-7.
6. Junaid TA and Odo EIA. Cytologic diagnosis of ascetic fluid in Ibadan; Nigeria. J Natl Med Assoc. 1980; 72(7): 669–72.
7. Hoda RS. Non-gynecologic cytology on liquid-based preparations: A morphologic review of facts and artifacts. DiagnCytopathol. 2007;35(10):621-34.
8. Linder J. Recent advances in thin-layer cytology. DiagnCytopathol. 1998;18(1):24-32.
9. Sakamoto H, Takenaka M, Ushimaru K, Tanaka T. Use of Liquid-Based Cytology (LBC) and Cell Blocks from Cell Remnants for Cytologic, Immunohistochemical, and Immunocytochemical Diagnosis of Malignancy," Open Journal of Pathology, 2012;2(3):58-65.
10. Thapar M, Mishra RK, Sharma A, Goyal V, Goyal V. Critical analysis of cell block versus smear examination in effusions. J Cytol. 2009; 26(2): 60–64.
11. Udasimath S, Arakeril SU, Karigowdar MH, Yelikar BR. The role of the cell block method in the diagnosis of malignant ascitic fluid effusions. Journal of Clinical and Diagnostic Research, 2012;(Suppl)-6(7):1280-3.
12. Yeoh GP and Chan KW. Cell block preparation on residual ThinPrep sample. DiagnCytopathol. 1999;21(6):427-31.
13. Grandhi B, Shanthi V, Rao NM, Reddy VC, Mohan K . The diagnostic utility of cell block as an adjunct to cytological smears. International Journal of Medical Research and Health Sciences 2014;3(2):278-84.
14. Karoo ROS, Lloyd TDR, Garcea G, Redway HD, Robertson GSR. How valuable is ascitic cytology in the detection and management of malignancy?PostgradMedJ2003;79:292–94.
15. Pradhan SB, Pradhan B, Dali S. Cytology of body fluids from different sites: an approach for early diagnosis of malignancy. J Nep Med Assoc 2006; 45: 353-356
16. Nathan NA, Narayan E, Smith MM, Horn MJ. Cell block cytology. Improved preparation and its efficacy in diagnostic cytology. Am J ClinPathol. 2000;114(4):599-606.
17. Santwani PM and Vachhani JH. Analysis of Diagnostic Value of Cytological Smear Method Versus Cell Blocks Method in Body Fluid Cytology: Study of 150 Cases. Ethiopian journal of health sciences 2014;24(2):125-30.
18. Kumavat PV, Kulkarni MP, Sulhyan SR. Cytological Study of Effusions. Indian Medical Gazette. 2013;306-313
19. Monte SA, Ehya H, Lang WR. Positive effusion cytology as the initial presentation of malignancy.ActaCytologica 1987;31(4):448-52.
20. Gabriel C, Achten R, Drijkoningen M. Use of liquid-based cytology in serous fluids: a comparison with conventional cytopreparatory techniques. ActaCytol. 2004;48(6):825-35.
21. Qing HE. Application of Liquid-based preparation cytology test in the research of pleural effusion and the ascites. Anhui Medical and Pharmaceutical Journal 2007;05.

How to cite this article: Mittal R, Kundal R, Singh H, Gupta D, Kaur B, Sandhu HS. Study of Ascetic Fluid by Liquid Based Cytology and Its Comparision with Conventional Cytosmears and Cell Block Preprations. Ann. Int. Med. Den. Res. 2016; 2(6):PT16-PT22.

Source of Support: Nil, **Conflict of Interest:** None declared