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Liquid Based Cytologyand Cell Block in Malignant Pleural Effusion – a Comparative Study

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ABSTRACT

Primary, metastatic and non-neoplastic pleural diseases can have similar clinical, radiographic and gross features. But treatment and prognosis vary greatly and so accurate diagnosis is important. Liquid based cytology (LBC) is a highly specific diagnostic tool but with limited sensitivity. Cell block (CB) and immunocytochemistry (IHC) can resolve this. This study compares sensitivity and diagnostic specificity of CB preparation versus LBC for detection of malignancy in fluid cytology, to assess usefulness of IHC and study frequency of occurrence of pleural diseases in studied population. A cross sectional observational study was carried out from January 2018 to December 2018 in the Department of Pathology, on 100 patients with pleural effusion. Along with LBC preparation, cell blocks were prepared using Plasma Thrombin clot method. Thyroid transcription factor 1(TTF-1), p63, p40 and calretinin immunostaining was used for further diagnosis. Majority of the samples were in age of 51–70 years. Male: female ratio was 1:1.18.Carcinoma of the breast was the most common primary in females followed by lung in males. Eight suspicious cases were subjected to IHC. On LBC preparation, 30 cases (41%) of pleural fluid effusion were positive for malignancy which on employing cell block increased to 36 cases (49.3%). The sensitivity and specificity of CB preparations compared to LBC preparation were 89% and 85% respectively. The CB and IHC staining should be integrated into LBC in clinical practice to improve diagnostic accuracy of pleural effusion.

KEYWORDS: Pleural effusion, liquid-based cytology, cell block, plasma-thrombin clot, immunohistochemistry

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INTRODUCTION

Primary, metastatic and non-neoplastic pleural diseases can have similar clinical, radiographic and gross features. But treatment and prognosis vary greatly and so accurate diagnosis is important. Pleural effusion is the most common symptom. Thoracentesisis a diagnostic procedure for patients with pleural effusion.[1]To differentiate between transudative or exudative causes and for the detection of unsuspected cancers and metastasis from cancer of unknown primary origin, pleural fluid examination has been accepted as a routine laboratory procedure.[2]Thediagnostic rates of cytology in malignant pleural effusion (MPE) range from 40% to 80%, whereas cell block preparations detect 85% to 87%.[3,4,5]

Liquid-based cytology (LBC) is accepted as routine practice in patients with advanced cancer.[3-5,7]LBC is a highly specific diagnostic tool but with limited sensitivity.[3.8]Differentiating reactive entity from malignancy needs extensive study of morphological features like architectural, nuclear and cellular details along with clinical and radiological findings. To increase the diagnostic accuracy of cytology, cell block (CB) method was developed which also allows to perform specialstains, immunocytochemistry (IHC).[9,10,11]Many benign reactive processes show significant atypical features mimicking malignant changes. Likewise, a few malignant condition slack sufficient atypical changes for a clear diagnosis of malignancy. With the advent of immunocytochemistry this problem can be largely resolved.

The objective of our study was to compare the sensitivity and diagnostic specificity of cell block preparation versus LBC technique for detection of malignancy in fluid cytology, to assess the usefulness of IHC and to study frequency of occurrence of various pleural diseases in the study population.

MATERIALS AND METHODS

A cross sectional observational study was carried out in Department of Pathology, Institute of Post Graduate Medical Education and Research, Kolkata for a period of 1 year from January 2018 to December 2018, on all patients >15 years of age admitted with pleural diseases (clinically and radio graphically) under Chest Medicine Department. Written informed consent was obtained from all patients before commencement of the procedure. Approval of the study was taken from the Institutional Ethics Committee. Necessary clinical data about the enrolled patients was collected as per proforma.

Pleural fluid cell count and Liquid Based Cytology

The procedure was performed within 60 min of receiving the fluid sample. Pleural fluid samples were sent in heparinized tubes. The physical characteristics of the fluid was examined for their appearance, color, and coagulum. Adenosine Deaminase (ADA) level was also considered. Total leukocyte count was obtained using the Neubauer modified counting chamber. Total leukocyte count was less than 300/µL and Light's criteria was applied to classify transudative and exudative effusion.

At first, the samples were processed to produce LBC slides using BD Sure Path™ following the manufacturer's protocol for sample preservation and slide preparation. Briefly,after fluid collection, samples were prepared bycytocentrifugation for 10 min at 2,000 rpm in Rotina-380.The supernatant was decanted off and the remaining pellet was treated with 5-10 ml of BD CytoRich™ preservation liquid. Using TriPath Imaging Multi Vial Vortexer, it was set to vortex at 60 minutes, followed by centrifugation for 10 minutes and the supernatant was decanted. Then a representative sample (1-5 drops) was transferred to 12 ml tube,10ml water was added and centrifuged for 5 minutes. The labelled 12 ml centrifuge tubes were loaded onto BD Prep Stain™slide processor for processing.

Cell Block study

About 15 mL of pleural fluid was centrifuged at 2000 rpm for 10 min. The supernatant was decanted off. The pellet was wrapped in filter paper. Coagulation of the pelletwas done by adding 2 – 3 drops of plasma and 3 – 4 drops of thrombin. It was placed in a cassette, embedded in paraffin, and cut and stained in the manner of histologic sections. The sections were reported by using light microscope at different magnification.

Immunohistochemistry

The suspicious specimens were subjected to immunohistochemistry (IHC) by peroxidase - antiperoxidase technique for further categorization. Calretinin [clone: polyclonal] was used for reactive mesothelial cells and thyroid transcription factor 1 (TTF-1) [Monoclonal Mouse Antibody; clone:8G7G3/1] was used to confirm the adenocarcinoma cells from ovarian (serous), breast, intestinal, and lung primary and p40 [Monoclonal Mouse Antibody; clone: ZR8], p63[Monoclonal Mouse Antibody;clone:4A4]for squamous cells. Proper controls for respective IHC stains were used and staining of tissues was assessed for nuclear or cytoplasmic positivity.

STATISTICAL ANALYSIS

Statistical analysis was done in Graph Pad In Stat 3.

RESULTS

100 cases of pleural fluid were included. Most common age group of the patients were 51 to 70 years with male to female ratio of 1:1.18.Most common symptoms include cough (52%) and shortness of breath (45%) which were followed by chest pain (2%) and fever (1%). Among the 100 cases, 25 were transudative and 75 were exudative.

Majority of pleural fluid were straw in color (54%) followed by hemorrhagic pleural effusions (40%). Pus was aspirated only in 5 cases (6%). During cell typing, lymphocytes were the most common cells found followed by polymorphs, mesothelial cells, and atypical mononuclear cells.

Of 75 cases of exudative pleural fluid cases, 2 were insufficient. So, 73 cases were included. As shown in Table1, on LBC35 cases(47.9%) were benign and 30 cases(41%) were malignant. The remaining 8 cases (10.9%) were suspicious for malignancy. On histological examination of cell block preparation,34 cases(46.6%) were benign followed by 36(49.3%) malignant cases and suspicious cases reduced to 3 (4.1%).Architectural patterns, such as glands, sheets (Figure 2B),three-dimensional cell clusters (Figure 3C) and cell balls were commonly observed in the CB method, whereas, singly scattered cells(Figure 1A) were predominant findings in LBC.

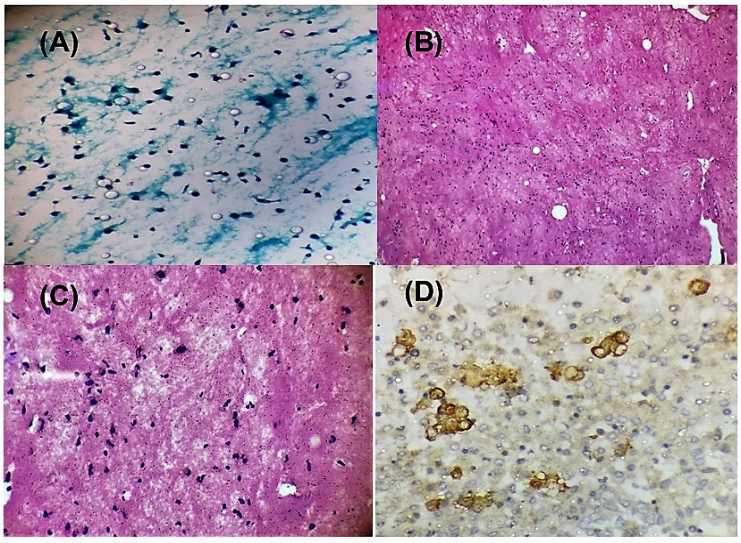

Figure 1:Cytologic, Histologic, Immunohistochemistry findings in a patient with Lymphocytic Pleural Effusion(A) LBC smear demonstrates lymphocytes (Papanicolaou staining, x400)(B) corresponding cell block section of the same patient (hematoxylin and eosin staining, x100) (C) corresponding cell block section of the same patient (hematoxylin and eosin staining, x400)(D) corresponding cell block section of the same patient showing calretinin positivity (x400)

Table 1: Analysis of discrepancies Liquid Based cytology and Cell Block technique in pleural fluid (N=73)

	Liquid based cytology		Cell block method	
	Number	Percentage	Number	Percentage
Benign	35	47.9	34	46.6
Suspicious	8	10.9	3	4.1
Malignant	30	41.0	36	49.3
Total	73	100	73	100

8 suspicious cases were subjected to immunohistochemistry using calretinin, p63 (Figure 2C), p40 (Figure 2E) and TTF-1 as shown in Table.2. 2 cases were confirmed with malignancy using TTF-1 (Figure 3D) and 2 cases (25%) were confirmed benign by calretinin (Figure 1D). IHC was done as supporting cell block finding in 4 cases (50%).

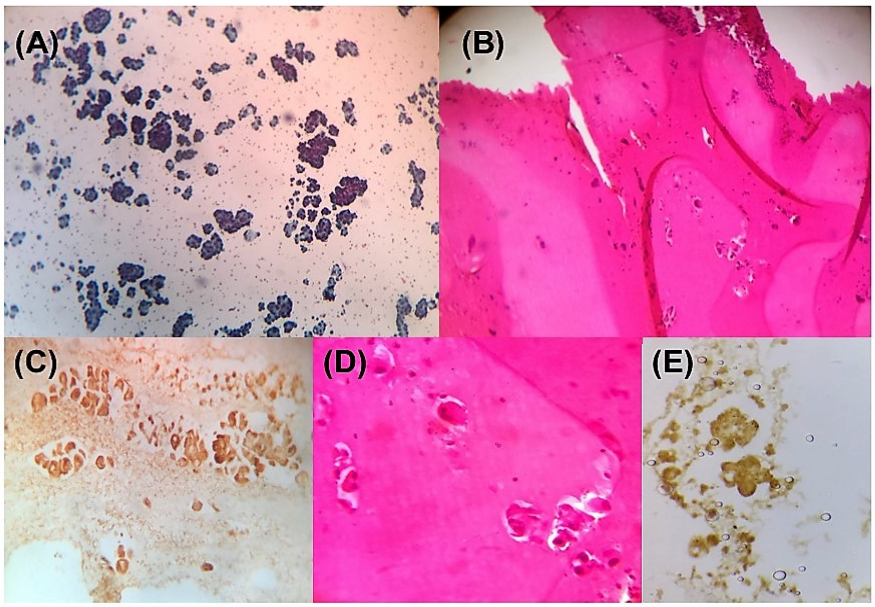

Figure 2: Cytologic, Histologic, Immunohistochemistry findings in a patient with Metastatic Pleural Effusion (Squamous Cell Carcinoma). (A) LBC smear demonstrates clusters of atypical cells (Papanicolaou staining, x400) (B) corresponding cell block section of the same patient (hematoxylin and eosin staining, x100) (C) corresponding cell block section of the same patient showing p63 nuclear positivity (x400) (D) corresponding cell block section of the same patient (hematoxylin and eosin staining, x400) (E) corresponding cell block section of the same patient showing p40 nuclear positivity(x400)

Table 2: Distribution of patients according to theirimmunohistochemistry findings(N=8)

Category	Number	p40	p63	calretinin	TTF-1	Percentage
Confirmed malignancy by IHC	2	-	-	-	+	25
Confirmed reactive by IHC	2	-	-	+	-	25
IHC done as supporting cell block finding	4	+	+	-	+	50
TOTAL	8					

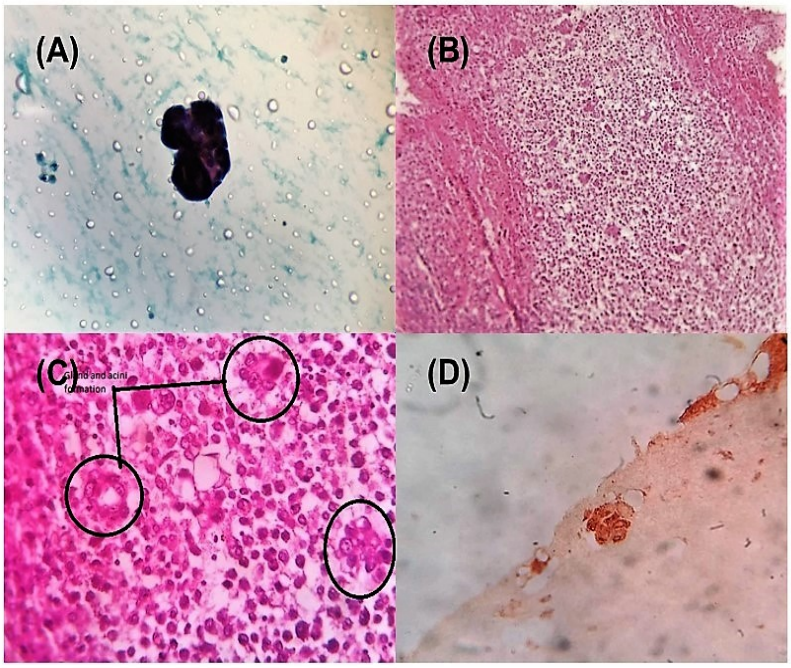

Figure 3: Cytologic, Histologic, Immunohistochemistry findings in a patient with MetastaticPleural Effusion (Adenocarcinoma). (A) LBC smear demonstrates metastatic adenocarcinoma with tumor cells arranged in three-dimensional configuration (Papanicolaou staining, x400)(B) corresponding cell block section of the same patient (hematoxylin and eosin staining, x100). (C) corresponding cell block section of the same patient (hematoxylin and eosin staining, x400). (D) corresponding cell block section of the same patient showing TTF-1 nuclear positivity(x400)

The sensitivity and specificity of CB preparations compared to LBC preparation for the detection of malignancy were estimated to be 89% and 85% respectively. Positive predictive value being 88.88% and negative predictive value being 86.95%. While comparing the LBC technique and CB methods, it was found that CB produced significantly better results (P = 0.03) while detecting malignant lesions and reducing suspicious results (P = 0.02).

DISCUSSION

Cytological examination of pleural fluids is a non-invasive technique. Thoracentesis to retrieve pleural fluid for examination is well accepted as an initial investigation. The cytological examination of serous effusions has increasingly gained acceptance in clinical medicine for the diagnosis of malignant lesions as well as staging and prognosis.[2]Malignant pleural effusion is a common complication of pulmonary and breast carcinomas. During thoracentesis, closed-blind pleural biopsy can be performed simultaneously to obtain pleural tissue for histology. But its diagnostic yield is less sensitive than LBC, as pleural metastases tend to be focal in the parietal pleura.[12,13]Pleural biopsy sometimes fails to provide adequate tissue.

In Liquid Based Cytology, two methods are well known, Thin Prep^{R} and BD Sure Path^{TM}. We have used BD Sure Path^{TM} for our study. Thin Prep Sample (TPS) method is a vacuum filtration method, whereas Sure Path Sample(SPS)method is a density-gradient centrifugation method. In SPS system, the cell-enrichment process substantially removes blood, mucus, and up to 50% of inflammatory cells from the diagnostic material, resulting in an enriched cellular sample, providing better visualization of clinically relevant cells. SPS with ethanol-based fixative provides cellular detail more familiar to thecytologist than the methanol-based fixative used by TPS.[14]Even though the area of the smear is smaller than in conventional preparations, SPS preparations are on an average more cellular with a cleaner background. Norimatsuet al., and Bentz et al., demonstrated that reduced cellularity on TPS slide glass from bloody samples results from blood and fibrin competing with epithelial cells and clogging the TPS filter pores.[14,15]Sweeney et al., and Kenyon et al., have shown that specimens processed in the SPS system had effectively no loss of cellularity with any amount of added mucus.[16,17]

The sediment from centrifuged pleural fluid can be processed as CB for histology, to enhance the diagnosis. In malignant pleural effusion diagnosis, LBC has certain advantages over conventional smear(CS). Features usually associated with CS such as thick, overlapping cellular areas, obscuring inflammation and blood and air-drying artifact result in poor cellular and nuclear preservation. CS are tedious and time-consuming to screen due to no uniform slide preparation and fixation.LBC is an automated process and increase the diagnostic accuracy.[18]But discriminating malignant cells among reactive mesothelialcells and macrophages in the pleural fluid is challenging using only the morphological features detected by LBC. However, CB has certain advantages provides better cellular morphological details, such as better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin and cellularity is high which is concentrated in one small area that can be evaluated at a glance.[19,20]

Although some studies have evaluated cell morphology and IHC performance of CB using different fixatives.[21,22]There is no consensus guideline in this process, leaving the choice up to each institute based on availability and cost-affordability. In this study, we have used the Plasma Thrombin clot (PT) method. Preparation of the PT block involved the least technical details. It was the simplest and the least time-consuming process and could be performed at room temperature as compared to HG (HistoGel) and CTC (CytoLyt-prefixed thrombin clot)method. In cell blocks prepared with the PT, cells tend to aggregate in the center of the clot and the cut sections. PT blocks revealed the best overall morphologic preservation with minimal artifacts. Cells in the HG sections exhibited exaggerated cytoplasmic vacuoles, denser cytoplasm, and more frayed cytoplasmic

borders, whereas cells in the CTC sections exhibited more cellular shrinkage and increased nuclear-cytoplasmic holes.[23]

In this study, due consideration was given to age, sex, site of effusion, clinical and radiological findings, to arrive at a final diagnosis and also to identify the primary malignant lesion. Maximum samples were in the age group of 51–70 years. Least number of patients were in the age group of 15–30 years. Male: female ratio was1:1.18. Similar findings were observed by Khan et al. and Dey et al.[19,24]The present study results for primary lesions were correlating with the Sears and Hajduand Johnstonstudies.[25,26]Sears and Hajdureported that the most common primary neoplasms causing pleural effusions were carcinoma of the breast (24%), followed by lung (19%) and lymph reticular system (16%), and in 15% of the cases the primary site was unknown. In our study for pleural fluid analysis, carcinoma of the breast (30%) was the most common primary followed by primary in the lung (25%) and gastrointestinal tract (20%) and in 10% of the cases the primary site was unknown. Most of the pleural effusions were of the adenocarcinoma type.

On routine cytological examination30 cases (41%) of pleural fluideffusion was reported positive for malignancy which on employing cell block increased to 36 cases (49.3%). An additional increase of six cases of malignancy contributing to 9.3% in the diagnostic yield was observed in our study by using cell block which is similar to findings of Bhanvadia VM et al., and Thapar M et al., who observedan additional increase of 14% and 10% respectively in diagnostic yield on employing cell block method in addition to routine cytologicalexamination.[27,28]We observed pericellular lacunae in more than 50% cases of adenocarcinoma cases considered to be helpful diagnostic feature in these cases.Price*et al*. also observed pericellular lacunae in the adenocarcinomacases in CB method.[29]

LBC and CB had no role in diagnosis of noninfectious inflammatory pleuritis and transudative effusion. Clinical manifestations and biochemical analysis are required to obtain a diagnosis in these conditions. In our study we performed immunohistochemistry in 8 suspicious cases.Several investigators have demonstrated that calretinin is a sensitive and specific marker for both benign and malignant mesothelial cells.[30,31]In this study, the reactive mesothelial cells of 2 benign cases demonstrated calretinin nuclear enhancement with strong membrane positivity**.**TTF-1 showed nuclear positivity in 4 cases thus confirming to be adenocarcinoma lung. 2 cases showed p63 and p40 nuclear positivity proved squamous cell carcinoma lung.

CONCLUSION

To conclude, analysis of CB preparations and TTF-1, p63, p40, calretinin immunostaining along with LBC showed a convincing diagnostic performance. Our present study results showed that the cellblock technique, by using plasma thrombin clot was a simple, inexpensive method, and did

not require any special training or instrument. The CB method yielded more cellularity and better architectural patterns which improved the diagnosis of malignancy by 9.3%. Multiple sections could be obtained if required for special stain or an Immunohistochemistry (IHC) study. Considering the synergistic effects, the CB method and IHC staining should be integrated into LBC in routine clinical practice to improve the diagnostic accuracy of pleural effusion, especially in cases in which malignancy is suspected or those showing equivocal cytological features.

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None

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCE

1. AssawasaksakulT, Boonsarngsuk V, Incharoen P. A comparative study of conventionalcytology and cell block method in the diagnosis of pleural effusion. J Thorac Dis2017;9(9):3161-3167.
2. Dekker A, Bupp PA, Cytology of serous effusions. An investigation into the usefulness of cell blocks versus smears. Am J Clin Pathol1978; 70:855-60.
3. Lee YM, Hwang JY, Son SM, et al. Comparison of diagnostic accuracy betweenCellprepPlus(R) and Thin Prep(R) liquid-based preparations in effusion cytology. Diagn Cytopathol2014; 42:384-90.
4. Hooper C, Lee YC, Maskell N. Investigation of aunilateral pleural effusion in adults: British ThoracicSociety Pleural Disease Guideline 2010. Thorax 2010;65Suppl 2:ii4-17.
5. Rivera MP, Mehta AC, Wahidi MM. Establishing the diagnosis of lung cancer: Diagnosis and management oflung cancer, 3rd ed: American College of Chest Physiciansevidence-based clinical practice guidelines. Chest2013;143: e142S-65S.
6. Zhao FH, Hu SY, Bian JJ, et al. Comparison of Thin Prep and Sure Path liquid-based cytology and subsequent humanpapillomavirus DNA testing in China. Cancer Cytopathol 2011;119:387-94.
7. Son SM, Koo JH, Choi SY, et al. Evaluation of Urine Cytology in Urothelial Carcinoma Patients: A Comparisonof Cellprep Plus(R) Liquid-Based Cytology and Conventional Smear. Korean J Pathol 2012;46:68-74.

8. Woo CG, Son SM, Han HS, Lee KH, Choe KH, An JY. Diagnostic benefits of the combined use of liquid-based cytology,cell block, and carcinoembryonic antigen immunocytochemistryin malignant pleural effusion. J Thorac Dis 2018;10(8):4931-4939.
9. Nathan NA, Narayan E, Smith MM, Horn MJ. Cytologyimproved preparation and its efficacy in diagnostic cytology. AmJ Clin Pathol 2000,114:599-606.
10. Kuhlmann L, Berghauser KH, SchafferR**:**Distinction ofmesothelioma from carcinoma in pleural effusions. Animmunocytochemical study on routinely processed cytoblockpreparations. Pathol Res Pract 1991;187:467-471.
11. Koksal D, Demirag F, Bayiz H, Koyungu A, Mutluay N, Berktas B, Berkoglu M. The Cell Block Method Increases the Diagnostic Yield in Exudative Pleural Effusions Accompanying Lung Cancer. Turk Patoloji Derg2013, 29:165-170.
12. 12.Prakash UB, Reiman HM. Comparison of needle biopsy with cytologic analysis for the evaluation of pleural effusion: analysis of 414 cases. Mayo Clin Proc1985.;60:158-64.
13. Nance KV, Shermer RW, Askin FB. Diagnostic efficacy of pleural biopsy as compared with that of pleural fluid examination. Mod Pathol 1991;4:320-4.
14. Norimatsu Y, Sakamoto S, Ohsaki H, Ozaki S, Yokoyama T, Shimizu K et al. Cytologic Features of the Endometrial Adenocarcinoma: Comparison of Thin Prep and BD SurePath Preparations. Diagn Cytopathol 41(8).
15. Bentz JS, Rowe LR, Gopez EV, Marshall CJ. The unsatisfactory Thin Prep Pap test: Missed opportunity for disease detection? Am J Clin Pathol 2002;117:457–463.
16. 16.Sweeney BJ, Haq Z, Happel JF, Weinstein B, Schneider D. Comparison of the effectiveness of two liquid-based Papanicolaou systems in the handling of adverse limiting factors, such as excessive blood. Cancer 2006; 108:27–31.
17. Kenyon S, Sweeney BJ, Happel J, Marchilli GE, Weinstein B, Schneider D. Comparison of BD Surepath and ThinPrep Pap systems in the processing of mucus-rich specimens. Cancer 2010; 118:244–249.
18. Hoda RS, Non-Gynecologic Cytology on Liquid-Based Preparations: A Morphologic Review of Facts and Artifacts. Diagn Cytopathol 35(10).
19. Khan N, Sherwani RK, Afroz N, Kapoor S. The cytodiagnosis of malignant effusions and determination of the primary site. J Cytol2005; 22:107-10.
20. Shivakumarswamy U, Arakeri SU, Karigowdar MH, et al. Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology. J Cytol2012;29:11-5.
21. Fetsch PA, Simsir A, Brosky K, et al. Comparison of three commonly used cytologic preparations in effusion immunocytochemistry. Diagn Cytopathol 2002;26:61-66.

22. 22.Nigro K, Tynski Z, Wasman J, et al. Comparison of cell block preparation methods for nongynecologic Thin Prep specimens. Diagn Cytopathol2007;35:640-3.
23. Jing X, Li QK, Bedrossian U, Michael CW. Morphologic and Immunocytochemical Performances of Effusion Cell Blocks Prepared Using 3 Different Methods. Am J Clin Pathol2013; 139:177-182.
24. Dey S, Nag D, Nandi A, Bandyopadhyay R. Utility of cell block to detect malignancy in fluid cytology: Adjunct or necessity? Journal of Cancer Research and Therapeutics2017;13:425-9.
25. Sears D, Hajdu SI. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. Acta Cytol1987;31(2);85-9.7.
26. Johnston WW. The malignant pleural effusion. A review of cytopathologic diagnosis of 584 specimens from 472 consecutive patients. Cancer1985;56(4):905-9.
27. Bhanvadia VM, Santwani PM, Vachhani JH. Analysis of Diagnostic value of cytological smear method versus cell block method in body fluid cytology: study of 150 cases. Ethiop J Health Sci 2014;24(2):125-39.
28. Thapar M, Mishra RK, Goyal V. Critical analysis of cell block versus smear examination in effusions. J Cytol2009;26:60-64.
29. Price BA, Ehya H, Lee JH. Significance of pericellular lacunae in cell blocks of effusions. Acta Cytol 1992;36:333-7.
30. Yahya ZM, Ali HH, Hussein HG. Evaluation of the sensitivity and specificity ofimmunohistochemical markers in the differential diagnosis of effusion cytology.Oman Medical Journal2013;28(6):410–16.
31. Murugan P, Siddharaju N, Habeebullah S, Basu D. Immunohistochemicaldistinction between mesothelial and adenocarcinoma cells in serous effusions: Acombination panel-based approach with a brief review of the literature. Indian JPathol Microbiol2009;52(2):175-81.