

“CELL BLOCK AS A BETTER DIAGNOSTIC TOOL IN DIAGNOSIS OF LUNG CANCER FROM EXUDATIVE PLEURAL FLUID”

Pathology

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ABSTRACT

Introduction: The cytological examination of serous effusions helps in diagnosis, staging, management and prognostication of patients with various malignancies. But cytological method has disadvantage of lower sensitivity in differentiating reactive atypical mesothelial cells from malignant cells.

Aim: The aim of this study is to compare the cytomorphology of pleural exudative fluids by conventional smear (CS) method and cell block (CB) method and also to assess the utility of their combined approach. Furthermore, immunohistochemistry is used in differentiating the benign from malignant tumour cells in exudative pleural effusions.

Materials and Methods: It is a cross sectional study. 38 pleural exudative fluid samples were subjected to evaluation by both CS and CB methods over a period of 1 year. Cellularity, architecture patterns, morphological features and yield for malignancy were compared. Chi-square and P-value were calculated, p value <0.05 considered as significant relationship.

Results: CB method provided higher cellularity, better architectural patterns and additional yield for malignancy as compared to CS method. p-value found to be <0.0318, and it is statistically significant.

Conclusion: The present study shows that it is advisable to routinely make CBs in every cases that were suspicious for malignancy by conventional smear examination method. Use of immunohistochemistry further aided in diagnosing and differentiating the benign cells from malignant cells in exudative pleural effusions.

KEYWORDS

Conventional smear method, Cell block method, Immunohistochemistry

INTRODUCTION

Pleural effusion is the accumulation of excessive fluid in the pleural space. The normal amount of pleural fluid in the body is 5-15 ml. Minimum 500 ml of pleural fluid is necessary for clinical detection. It may develop when there is excess pleural fluid formation from the interstitial spaces of the lung, the parietal pleura or the peritoneal cavity, or when there is decreased removal of the fluid by the lymphatics¹.

The leading causes of exudative pleural effusions are malignancy, bacterial pneumonia, viral infection and pulmonary embolism¹. Chest ultrasound has replaced the lateral decubitus x-ray in the evaluation of suspected pleural effusions and as a guide to thoracentesis.

Lung cancer is one of the most common malignancies with high mortality worldwide. It is generally a disease of the older adults, occurring most frequently between ages 55 and 84 years, with a peak incidence between 65 and 74 years. Only 2% of all cases occur before the age of 40³. In patients with lung cancer, the incidence of pleural effusion ranges between 7% and 23%.

The majority of the lung cancers among non smokers occur in women. The factors which are responsible are- infections, hormones, geographic, cultural and genetic factors.

All histological types of lung carcinomas are likely to cause pleural effusion. The relative frequencies of the various microscopic types of lung carcinoma have changed over the years. Adenocarcinomas are now the most common histological type of non squamous cell lung carcinoma while both squamous cell and small cell carcinoma incidence rates have declined.

Adenocarcinomas comprise over half of the lung carcinomas in females and 45% in males. Adenocarcinomas possess glandular differentiation or mucin production and may show acinar, papillary, lepidic, or solid features or a mixture of the patterns. About 65% of the cases have peripheral location and often invade the visceral pleura at the time of excision, which results in puckering and retraction of the pleura⁴.

There may be mixtures of histologic patterns even in some cancers. Squamous cell carcinoma is more common in men and strongly associated with smoking. It may involve the bronchus leading to obstruction, atelectasis and infection. Small cell carcinoma are the most aggressive of lung tumours, arising in the major bronchi or in the periphery of the lung. Among the various lung tumours, small cell

carcinoma is most commonly associated with ectopic hormone production. Large cell carcinoma is an undifferentiated malignant epithelial tumour. Large cell carcinoma is a diagnosis of exclusion since it does not express the markers associated with adenocarcinoma or squamous cell carcinoma.

Malignant pleural effusion can occur secondary to metastatic disease. It is the second most common type of exudative pleural effusion. The three tumours that cause 75% of all malignant pleural effusions are lung carcinoma, breast carcinoma and lymphoma¹. The diagnosis usually made by cytological examination of pleural fluid.

Cytological examination of serous fluids is one of the commonly performed investigations. It has been universally recognized as the most important diagnostic tool in the recognition of malignant tumours in effusions. It assists the clinician in formulating and pointing out the etiology of effusions and list of differential diagnosis. The cytodiagnosis by conventional smears have got lower sensitivity due to overcrowding of cells, cell loss and different laboratory processing methods⁵.

Various studies have shown that the cytological examination of effusions 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 very useful in increasing diagnostic yield by Cell Block method. The Cell Block (CB) technique is one of the oldest and complementary methods for the evaluation of body effusions. The CB preparation concentrates the cells in a limited field without the loss of cellular material and tissue architecture and further utilizes this for routine as well as ancillary tests to resolve diagnostic problems. The major advantage of CB is the availability of multiple sections of the same material. From cell block, we can go for further ancillary techniques to diagnose malignancy causing pleural effusion.

CB is of particular value in effusion samples subjected to immunohistochemical staining (IHC) to elucidate the occult primary site of tumour. Besides primary diagnosis, IHC test on cell block is also useful for prognostication. Immunoreactivity for pneumocyte markers, most commonly TTF-1 and/or Napsin A is sufficient to establish the diagnosis of adenocarcinoma in poorly differentiated carcinomas that lack glandular histology or mucin secretion. TTF-1 is a tissue specific gene expression marker that has been reported to play a crucial role in the molecular pathogenesis of primary adenocarcinoma of the lungs. It has been observed in 70% of pulmonary adenocarcinomas⁶.

Thus this present study has been undertaken to assess the utility of cell block preparation method in increasing the sensitivity of cytodiagnosis of adenocarcinoma of lung from exudative pleural effusion.

MATERIALS AND METHODS-

The present study was conducted on 38 patients who underwent pleural tapping for the diagnosis of effusion cytology by conventional method and cell block methods. It is a hospital based cross sectional study. A total of 38 specimen(pleural fluid) were received in the Cytology section, Department of Pathology, Gauhati Medical College and Hospital, Guwahati, from December 2019 to November 2020. Written informed consent of all the patients in the study was obtained. Clotted fluid samples, time between collection and processing more than one hour were excluded from our study.

Ten milliliters of each fresh fluid specimen was divided into two equal parts of five milliliters each. One part was subjected to the conventional smear cytology technique and the other was subjected to cell block technique.

Conventional smear technique-

The five milliliter sample was centrifuged at 2500 rpm for 15 minutes. Two thin smears were prepared from the sediment. One smear was prepared after air drying and stained with the May-Grunwald-Giemsa stain. The other smear was immediately fixed in 95% alcohol and stained with Papanicolaou stain.

Cell block method-

The other five milliliter sample that remained was subjected to centrifugation at 2500 rpm for 15 minutes. The supernatant was poured off and one drop of pooled plasma and two drops of thromboplastin were added to the sediment and centrifuged again at 2500 rpm for 3 minutes for proper aggregation of the sediments. Then the sediments were put in a filter paper, wrapped and placed in 10% formalin for 8 hours for fixation. These are then processed in histokinette as part of routine paraffin- embedded section preparation. Paraffin embedded Cell block sections were stained by Hematoxylin and Eosin stain. Sections were mounted by DPX mountant and examined after drying.

The conventional smears and cell block sections were examined separately for cellularity, architectural patterns and morphology (cytoplasmic and nuclear details) to come at a cytological diagnosis for each case and the findings of each case were compared. The cases were diagnosed by clinical history, laboratory tests, radiological investigations, cytological examination, cell block technique and subsequently each were categorized as Benign, Suspicious for malignancy and Malignant pleural effusion particularly Adenocarcinoma of lung. Yield for malignancy were identified by both the conventional method and the cell block method. Data was entered into Microsoft excel 2007 sheet and analysed. Proportion and Chi-square test statistic were calculated. To find out whether the results were significant or not, χ^2 was used. Assessment and comparison between both the conventional method and cell block methods were carried out.

RESULT

This study was conducted among 38 patients admitted in Gauhati Medical College and Hospital, Assam. The means of diagnosis was pleural fluid cytology.

SEX DISTRIBUTION

Out of 38 patient, 29 patient were male and rest 9 were female.

TOTAL NO OF PATIENT	MALE	FEMALE
38	29(76.31%)	9(23.69%)

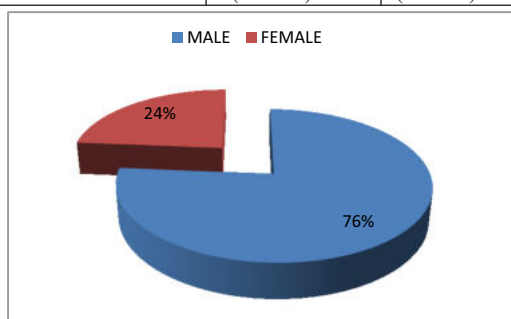


Fig 1-SEX DISTRIBUTION

AGE DISTRIBUTION

Patients included in the study were age ranging from 35 year to 94 years.

Most commonly affected age group is 65-74 year group and least affected group were 35-44 year group.

AGE GROUP(IN YEAR)	PATIENT NO
35-44	1
45-54	3
55-64	8
65-74	14
75-84	10
85-94	2

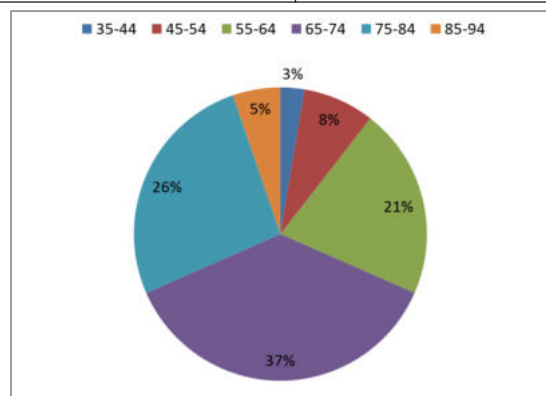


Fig 2-AGE GROUP DISTRIBUTION.

DIAGNOSTIC ACCURACY OF CS AND CB.

Cellularity was more by the CB method when compared to the CS method. Architectural patterns, such as, glands, sheets, three dimensional cell clusters and cell balls and papillae were commonly observed in the CB method, whereas, singly scattered cells were predominant findings in CS.

After analysis of the above samples, they were categorized as benign(BEN), suspicious for malignancy(SUM), Malignant sample like adenocarcinoma lung(ADL), Squamous cell carcinoma lung(SCL), small cell carcinoma lung(SML) and Large cell carcinoma lung(LCC). By the cell block method, an additional 10 cases of lung malignancies were detected {8 ADL, 1 SCL, 1 SML}. Yield is 26.31% more than the CS method and independent yield for adenocarcinoma lung were 21% out of 26.31% yield. These samples were reported as either suspicious for malignancy or benign samples. Out of the 11 samples that were reported as suspicious for malignancy in CS, seven(7) samples were diagnosed as ADL, one(1) SCL by the CB method. But 3 samples are found to be suspicious of malignancy even with CB method. Also from 6 samples of benign cases, additional 1 ADL and 1 SML were detected and 4 samples remained as benign even in the CB method. Use of immunohistochemistry, TTF-1(after ruling out primary thyroid neoplasm) further aided in diagnosing and differentiating the benign cells from malignant cells in exudative pleural effusions.

Out of 25 adenocarcinoma lung, TTF1 positive found in 8 cases, and it consolidate the diagnosis.

Out of 25 adenocarcinoma lung, 19 were male and 6 were female patients.

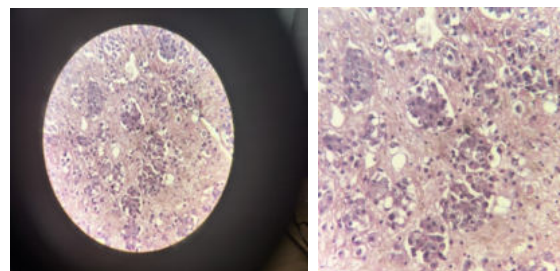


IMAGE1-Photomicrograph showing adenocarcinoma in Cell Block (H and E, 40X) with highly pleomorphic cells arranged in multiple clusters, small papillae and some singly dispersed cells.

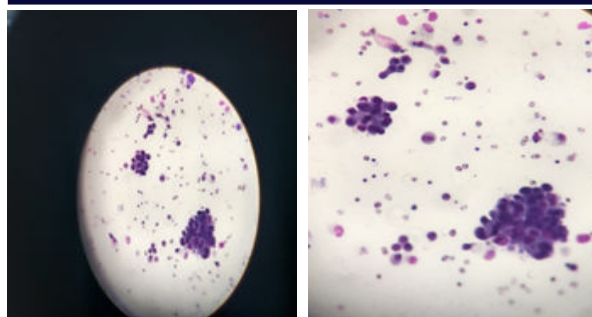


IMAGE 2-Photomicrograph showing adenocarcinoma in conventional smear (MGG Stain, 40X) with multiple clusters and singly dispersed atypical pleomorphic cells.

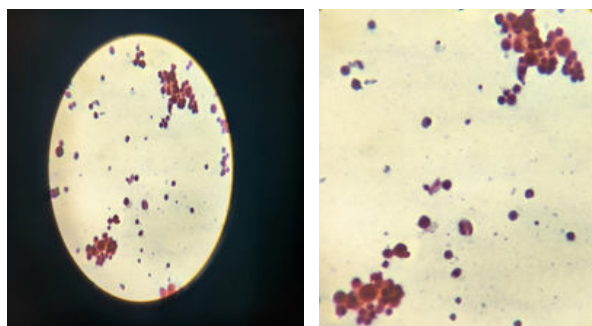


IMAGE3-Photomicrograph showing adenocarcinoma in conventional smear (PAP Smear, 40X)

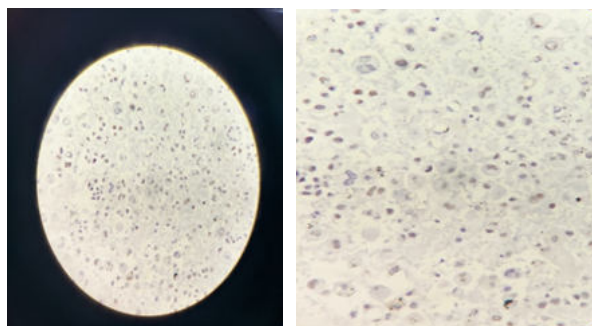


IMAGE 4-Photomicrograph showing TTF1 expression in adenocarcinoma lung in Cell block (40X) with nuclear staining

The CS and CB finding of the detail microscopic study is presented in the below mentioned tables.

Table 1- Showing Microscopic finding of CS Vs CB method in exudative pleural fluid.

Method	Malignant lung cancer	Suspicious of malignancy	Benign
CS	21	11	6
CB	31	3	4

The chi-square statistics is 6.8945. The p-value is <0.0318. The result is statistically significant at $p < 0.05$. (using 5x5 table or less).

METHOD	ADL	SCL	SML	LCC	SUM	BEN
CS	17	2	1	1	11	6
CB	25	3	2	1	3	4

Comparison of accuracy of CS Vs CB for diagnosis of lung malignancy

Table2-Showing microscopic finding of CS and its accuracy in CB method.

CONVENTIONAL SMEAR METHOD						CELL BLOCK METHOD					
MLC						MLC					
ADL	SCL	SML	LCC	SUM	BEN	ADL	SCL	SML	LCC	SUM	BEN
17	0	0	0	0	0	17	0	0	0	0	0
0	2	0	0	0	0	0	2	0	0	0	0

0	0	1	0	0	0	0	0	1	0	0	0
0	0	0	1	0	0	0	0	0	1	0	0
0	0	0	0	7	0	7	0	0	0	0	0
0	0	0	0	1	0	0	1	0	0	0	0
0	0	0	0	3	0	0	0	0	0	3	0
0	0	0	0	0	4	0	0	0	0	0	4
0	0	0	0	0	1	1	0	0	0	0	0
0	0	0	0	0	1	0	0	1	0	0	0
=17	=2	=1	=1	=11	=6	=25	=3	=2	=1	=3	=4

(MLC-Malignant lung cancer, ADL-Adenocarcinoma lung, SCL-Squamous cell carcinoma lung, SML-Small cell carcinoma lung, LCC-large cell carcinoma lung, SUM-Suspicious of malignancy, BEN-Benign)

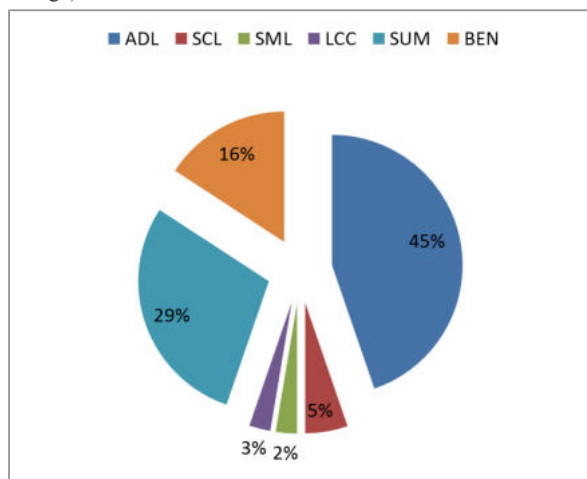


FIG 3- Various lung malignancies in CS

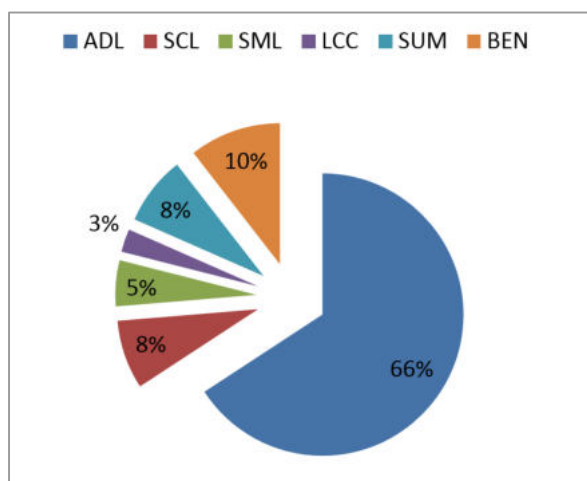


FIG 4- Various lung malignancies in CB

DISCUSSION

The cytological study of serous effusions is one of the most definitive diagnostic tool and has growingly gained acceptance in clinical and diagnostic medicine. Apart from diagnosis of malignancy, it also helps in staging and prognosis of various malignancies.

Occurrence of malignant pleural effusion is one of most important features of Adenocarcinoma lung and other lung malignancy.

In this study using exudative pleural fluid, CB method yields more adenocarcinoma lung than CS. Out of the 38 cases by CS method, 44.73 % (17) found to be adenocarcinoma lung. By CB method, 65.78% (25) found to be adenocarcinoma lung. So yield is 21 % more in CB method for adenocarcinoma lung. Use of immunohistochemical marker, TTF1 further added in differentiating the benign cells from malignant cells in exudative pleural effusion.

Ceyhan Ugurluoglu, Ercan Kurtipek et al⁷ found that the cell block method applied in exudative pleural fluid study, yield of adenocarcinoma

lung is more than any other lung and other carcinoma. This supports our study where, by the cell block method, 25 cases are found to be adenocarcinoma lung out of 38 cases, compared to 17 cases out of 38 cases by conventional method.

Bista P⁸ in their study “Comparison of the diagnostic accuracy of cell block with cytology smear in serous effusions” conclude that Cell block preparation is simple, rapid and inexpensive technique for serous fluids in which malignant cells can be reliably detected. Similarly in our study, it is seen that routinely making cell blocks in every cases that were suspicious of malignancy or benign by conventional smear method, the cell block method and use of IHC increases the yield of diagnosing and differentiating the benign cells from malignant cells like adenocarcinoma lung in exudative pleural fluids. It can be a promising, cost effective, easily available tool for adenocarcinoma lung or any other lung malignancy.

In our study, cellularity, architecture pattern, morphological features and yield for malignancy were compared between the conventional smear method and cell block methods. The cell block method increases the diagnostic yield in exudative pleural effusions due to lung cancer by 21%. **In the similar study by Hücre Bloğu Yöntemi, Akciğer Kanseri et al⁹**, concluded that the cell block method combined with conventional smear increases the diagnostic yield in exudative pleural effusions accompanying lung cancer.

Bhanvadia Viral M, Santwani P.M et al¹⁰ from their study on “Analysis of diagnostic value of cytological smear method versus cell block method in body fluid cytology – a study of 150 cases”, found that for the final cytodiagnosis of body fluid, there is statistically significant difference between the two techniques. In other words, CB is superior to CS method. This supports our study where in the conventional smear method, there are reactive mesothelial cells, inflammatory cells and a paucity of representative cells, lack of tissue architecture which lead to difficulties while making a conclusive diagnosis. In our study, 11 cases were suspicious of malignancy by CSS method. By CB method, only 3 cases were suspicious of malignancy, while 8 cases were actually malignant. 6 cases were detected as benign by CSS method. By CB method, 4 cases were concluded as benign while the remaining 2 were malignant. So yield for malignancy is more by CB method.

Sandeep S. Matreja, Kamal Malukani et al¹¹ in their study found out that architectural patterns, such as cell balls, sheets, cells cluster, glands and papillae were better observed in CB as compared to CS (p value < 0.005). Again in CS method, 4 cases was malignant effusion but in CB method, 13 cases were found to be malignant effusion. These findings support our present study, where we came to a conclusion that Cell Block method provided higher cellularity, better architectural patterns and additional yield for malignancy as compared to CS method.

Shivakumarswamy, et al.¹² from their study “Diagnostic utility of cell block method versus the conventional smear study in pleural fluid cytology” derived that, the CB method provides high cellularity, better architectural patterns, morphological features and an additional yield of malignant cells, and thereby, increases the sensitivity of the cytodiagnosis when compared with the CS method. This supports our study, where apart from better morphological details and architecture, preservation of the architectural patterns such as cell balls, papillae and three dimensional clusters, better nuclei and cytoplasmic preservation were obtained. All these features increased the sensitivity of the diagnosis of malignancies by CB method.

Sumedha dey, Dipanwita nag et al¹³, in their study found that from exudative pleural fluid study by CS method, they found 7(26.92%) cases of malignancy as compared to CB method, where they found 11(42.30%) cases. Additional 4(15.38 %) yield of malignancy detected by CB method. This study again support our study where we got additional 26.31% yield for lung malignancy using CB method.

CONCLUSION

The present study shows that it is advisable to routinely make CBs in every cases that were suspicious of malignancy or benign by conventional smear examination method. Use of immunohistochemistry further consolidate in diagnosing and differentiating the benign cells from malignant cells like adenocarcinoma lung in exudative pleural fluids. It can be a promising, cost effective, easily available diagnostic tool for

adenocarcinoma lung or any other lung malignancy.

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