



A STUDY OF PLEURAL EFFUSION BY COMBINED APPROACH OF CONVENTIONAL SMEAR AND CELL BLOCK TECHNIQUE

Pathology

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ABSTRACT

BACKGROUND: Cytological examination of pleural fluid is of paramount importance not only for diagnosis, staging, prognostication and management of malignancy also gives information about various inflammatory and non-inflammatory conditions. Conventional smear method has some disadvantages which can be overcome by cell block technique. **AIMS AND OBJECTIVES:** To compare cytomorphological features of conventional smears versus cell block technique and also to evaluate the diagnostic efficacy of a combined approach of conventional smear study and cell block technique. **MATERIAL AND METHOD:** 150 Sterile fluid sample were processed in cytology section, Department of Pathology, DECCAN COLLEGE OF MEDICAL SCIENCES, HYDERABAD. This study was conducted over a period of one and half years from January 2018 to June 2019. Each sample was divided into two equal parts one part was subjected to conventional smear method and other part was subjected to cell block technique. Statistical analysis was done using Microsoft Excel to calculate sensitivity and specificity of conventional cytospins and cell block technique. **RESULT:** Sensitivity and additional yield for malignancy was 50% more by cell block method.

KEYWORDS

Conventional smears(CS), Cell Block(CB).

INTRODUCTION:

Cytological examination of pleural fluid is of paramount importance not only for diagnosing cancer but they reveal information about inflammation regarding various inflammatory conditions, parasitic infestation, infection with bacteria, fungi, viruses and some immunological conditions. Cytological examination also helps for staging and prognosis of diseases. It is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as prognosis of disease.¹ An accurate identification of cells as being either malignant or reactive mesothelial cells in serous effusions is a common diagnostic problem in conventional cytological smears (CS).² Distinguishing benign from malignant cellular changes may require meticulous screening, careful scrutiny of cellular features and an understanding of the range of reactive changes. Cellular overlapping, delaying artifact, suboptimal processing, preparatory cytotechnique and leaving behind useful material causes lower diagnostic yield in cytology smears method. This residual material can be very useful in increasing diagnostic yield by the cell block method. The cell block technique is one of the oldest and complementary methods for the evaluation of body cavity fluids. Cell block preparation increases the sensitivity of detecting malignancies, and also has the ability to reduce false positive interpretations.³

AIMS AND OBJECTIVES

To compare cytomorphological features of conventional smears versus cell block technique and also to evaluate the diagnostic efficacy of a combined approach of conventional smear study and cell block technique.

MATERIALS AND METHODS

This study on pleural fluid cytology and cell block technique was undertaken in cytology section, Department of Pathology, DECCAN COLLEGE OF MEDICAL SCIENCES, HYDERABAD. This study was conducted over a period of one and half years from January 2018 to June 2019. This study was prospective study and was approved by Institutional Ethical Committee of DECCAN COLLEGE OF MEDICAL SCIENCES.

150 Sterile fluid sample were received in the cytology section, is processed maximum within one hour of receiving the sample. Simultaneously the clinical data with relevant radiological and laboratory information is collected. Gross examination for coagulum is done. If present, removed and sent for cell block. The fluid was divided into two equal volumes. The first volume was used for conventional cytospin, where the fluid was centrifuged in Shandon Cytospin 4 at 800 rpm for 3 minutes, the supernatant was discarded and eight smears prepared from the sediment. Four were air dried (two thick & two thin smears) and stained with Leishman stain and four smears (two thick & two thin smears) were wet fixed in 95% alcohol and stained with Hematoxylin-Eosin stain for conventional smear cytology.

The second volume of fluid was used for preparation of cell block preparation. The fluid was centrifuged in Shandon Cytospin 4 at 800 rpm for 3 minutes, the supernatant was discarded and the sediment completely drained off. The pellet formed was removed with pointed spatula at fixed in 10% neutral buffered formalin. The clot was cut into two fragments, were all embedded in the tissue cassettes and processed as other tissues in histopathology. The cell block are processed in Yorko automatic tissue processor manufactured by York Scientific Industries Pvt, Ltd to cover a series of steps, which include fixation, dehydration, clearing & infiltration to ensure sections of optimal diagnostic quality. Multiple thin sections of 3 to 5 micron thickness were cut using a rotary microtome (Leica) & stained with Hematoxylin-Eosin stain and later studied under our departmental LABOMED (Lx 300) microscope.

INTERPRETATION OF CONVENTIONAL CYTOSMEARS AND CELL BLOCK

The morphological criteria such as cellularity, architectural pattern (singly scattered, Cell balls, Clusters, Papillae, Glands & sheets), cytoplasmic and nuclear features details were used for giving cytological diagnosis.

CELLULARITY

The cellular material in conventional smear was considered to be mild when 5-50 nucleated cells per high power field, moderate when there were 50-200 cells per high power field & marked when there were more than 200 cells per high power field. The cellular material in the cell block was considered to be mild when 5-200 nucleated cells per high power field, moderate when there were 200- 1000 cells per high power field & marked when there were more than 1000 cells per high power field.

ARCHITECTURAL PATTERNS

The architectural pattern closely reflects histology, there by diagnosis was obvious. Architectural pattern was noted eg; singly scattered, Cell balls, Clusters, Papillae, Glands & sheets.

After studying all the available clinical, cytological and histopathological data, the cases were categorized as benign, suspicious, and malignant lesions. All the infectious and noninfectious inflammatory diseases involving pleura are included in benign category. In conventional smears the identification of reactive and atypical mesothelial cells is a diagnostic problem, those cases are included in suspicious for malignancy category. The cases with atypical cytology features of malignancy were included in malignant category.

Statistical analysis was done using Microsoft Excel to calculate statistical significance, sensitivity and specificity of conventional cytospins and cell block technique.

RESULTS

In the present study, 150 pleural fluid samples were evaluated by combined approach of conventional smear cytology and cell block technique from January 2018 to June 2019, in cytology section, Department of Pathology, DECCAN COLLEGE OF MEDICAL SCIENCES, Hyderabad.

Out of 150 pleural fluids, 79 cases (53%) were male and 71 cases were female (47%). Male: female ratio was 1.12:1. (Table-1)

Out of 150 pleural fluids, maximum number of samples i.e., 28 cases were in the age group of (61-70) Yrs. Least number of samples were in the age group of (1-10) yrs. In males predominantly i.e., 18 cases were in the age group of (61-70) yrs. In females predominantly i.e., 14 cases were in the age group of (51-60) yrs. Youngest patient was of 9 years and oldest patient was of 89 years.

Exudative effusions were more (91 %) compared to transudative effusions (9%).

By conventional smear cytology, predominantly there was mild cellularity was observed in 87 samples (58%), whereas by cell block technique the cellularity increased in 17 cases out of 87 cases. (Table-2)

By conventional smear cytology, benign features were observed in 139 cases (92.6%), 5 cases (3.33%) were suspicious for malignancy and 6(4%) cases were positive for malignancy. By cell block technique, benign features were observed in 139 cases (92.6%) and 12 cases (8%) were positive for malignancy. (Table-3).

In the benign effusions, various clinical conditions noted, predominantly 106 cases (76.8%) were of pulmonary tuberculosis, followed by pneumonia, congestive cardiac failure, anemia, cirrhosis and each case of pneumothorax, endometriosis and pulmonary infarction.

Out of 150 pleural fluid cases discrepancies noted in 6 cases. By conventional smear cytology one case was benign and five cases were suspicious of malignancy. By cell block preparation one benign case diagnosed by conventional smear method turned out to be malignant and five suspicious cases turned out to be malignant. Another 2 cases which were clinically and radiologically suspicious of lung carcinoma, their effusion was found to be inflammatory due to concomitant infection. (Table-4)

Out of 12 malignant effusions, the most common primary site was lung. Females had one each case of Primary site of breast, ovary carcinoma and mesothelioma. Both male & female had one each case of primary lung carcinoma. Males had preponderance in primary of GIT when compared to females. Females had predominance of malignant effusions (67%) compared to males (33%) with M:F ratio of 1:3. Additional yield for malignancy was 50% more by cell block method when compared to conventional smears. (Table-5)

Statistical analysis showed that sensitivity and specificity of cell block study in diagnosing malignant lesions were 100% where, 50% & 100% respectively by conventional smear method.

Table-1 Distribution Of Pleural Effusion According To Sex

SEX	NO OF SAMPLES	PERCENTAGE
MALE	79	53%
FEMALE	71	47%
TOTAL	150	100%

Table-2 Comparison Of Cellularity In Conventional Cytosmears (CS) And Cellblock (CB) Technique

Cellularity	CS		CB	
	NO	%	NO	%
Mild	87	58	70	46.66
Moderate	45	30	55	36.66
Marked	18	12	25	16.66
Total	150	100	150	100

Table-3 Distribution Of Pleural Fluid Analysis Based On Morphological Diagnosis

FEATURE	CS		CB	
	NO	%	NO	%
Benign(inflammatory)	139	92.67	138	92

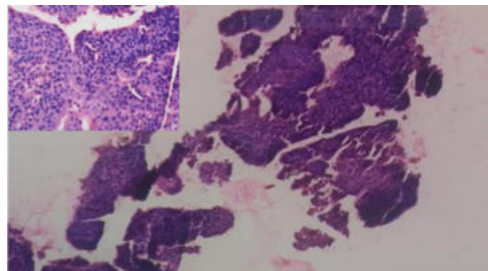
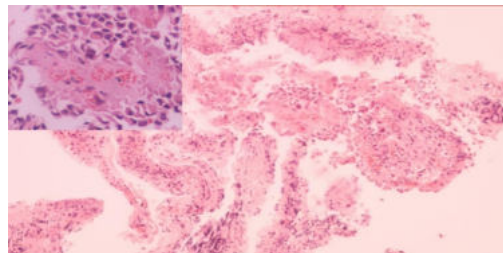
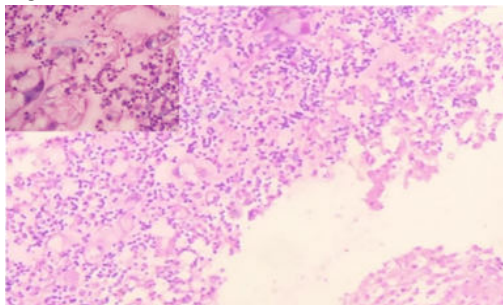
Suspicious	05	3.33	0	0
Malignancy	06	4	12	8
Total	150	100	150	100

Table-4 Analysis Of Discrepancies Between Clinical Diagnosis And CS & CB of Pleural Fluid

Clinical diagnosis	
Benign(inflammatory)	142 (94.67%)
Malignant	08 (5.33%)
Conventional smear	
Benign	139 (92.67%)
Suspicious	05 (3.33%)
Malignant	06 (4%)
Cell block technique	
Benign	138 (92%)
Suspicious	0 (0%)
Malignant	12 (8%)

Table-5 Distribution Of Primary And Metastatic Pleural Effusions In Male And Female In Malignant Effusions

Sl no	Primary site	Malignant effusion		
		M	F	Total
1	Mesothelioma	00	01	01(08%)
2	Ovary	00	01	01(08%)
3	Breast	00	01	01(08%)
4	Lung	01	01	02(17%)
5	GIT	01	00	01(08%)
6	Unknown	02	04	06(50%)
7	Total	04	08	12(100%)

**Fig 1:** Showing Cell Block Image Of Breast Carcinoma(10x H&E); Inset Showing 40x.**Fig 2:** showing cell block image of mesothelioma(10x H&E); Inset showing 40x**Fig 3:** Showing Cell Block Image Of Adenocarcinoma Colon (10x H&E); Inset Showing 40x**DISCUSSION**

In the present study of 150 cases, predominantly 28 cases were in the age group of 61-70 years. In males, predominantly, 18 cases were in the age group of 61-70 years, while in females predominantly 14 cases were in the age group of 51-60 years. While in study by Grandhi et al⁴,

predominantly i.e., 55 cases were in the age group 51-60 years. In males, predominantly 25 cases were in the age group of 41-50 years & in females 38 cases were in age group 51-60 years.

In the present study, males had more incidences (53%) compared to females (47%). Shobha et al⁵ in 2012, Grandhi et al⁶ 2014, Prashant et al⁶ in 2015 & Priyanka et al⁷ in 2017 showed more incidence of pleural effusion in males compared to females. Sherwani R et al⁸ in 2005 & Khan et al⁹ in 2005 in their study had more incidence of females compared to males in pleural effusion.

Analysis of effusion often gives an indication of underlying pathophysiological process. In present study most of effusions were exudative in nature (91%) and only 9% transudative. Light RW et al¹⁰, Shobha et al⁵ & Dey et al¹¹ also had an increase incidence of exudative compared to transudates effusion.

In the present study of 150 samples, by conventional smear cytology, mild cellularity was observed in 87 samples (58%), moderate cellularity in 45 samples (30%) and marked cellularity in 18 samples (12%). By cell block preparation mild cellularity was observed in 70 samples (46.6%), moderate cellularity in 55 samples (36.6%) and marked cellularity in 25 samples (16.6%). Similar findings were noted in study by Udasimath et al¹².

By conventional cytosmears, 139 cases (92.66%) were benign (inflammatory), 5 cases (3.33%) were suspicious for malignancy and 6 cases (4%) were positive for malignancy and by cell block technique, 138 cases (92%) were benign (inflammatory) and 12 cases (8%) were positive for malignancy in present study. 5 cases which were given as suspicious of malignancy and 1 case which was given benign in conventional smears were proved as malignant effusions in Cell block technique. Cell block is more useful in pleural effusion cases suspicious of malignancy compared to inflammatory effusions. Our study is in concordance with Bodele et al¹³ and Saraswathi et al¹⁴.

Effusions in cancer patients are not always "malignant" but may be reactive and of transudative type. In cancer cachexia, hypoproteinemia due to cancer associated malnutrition, external pressure on vascular or lymphatic channels or the effusion may be exudative due to concomitant inflammatory disease. In these cases the cancer cells are not found in pleural effusion.

In the present study there were 2 known cases of lung carcinoma with pleural effusion, whose diagnosis was made by histopathology. The pleural fluid was exudative and inflammatory due to concomitant tuberculosis. There was no evidence of cancer cells. Similarly, in a study by Thaper et al¹⁴, there were 70 cases of body fluids of cancer patients. By conventional smear method cancer cells were yielded in 50 cases & cell block further increased the diagnostic yield and showed positivity in 60 cases. 12 cases (8%) were diagnosed as malignant effusions & 138 as benign (inflammatory), based on clinical data and cytomorphological features the diagnosis was made. More number of cases were of pulmonary tuberculosis in 109 cases followed by pneumonia in 18 cases & least were from pulmonary infarction and endometriosis. While in a study by Udasimath et al¹² and Shobha et al⁵, the diagnosis of pulmonary tuberculosis was made in 17 & 52 cases respectively. Least number of cases in study of Udasimath et al¹² and Shobha et al⁵ were of congestive cardiac failure and pulmonary infarction respectively.

In the present study, 12 cases (8%) were of malignant effusions, predominantly were females (66.67%) when compared to males (33.33%). Out of 12 malignant effusions, primary was known in 6 cases (50%), which includes 2 cases of lung carcinoma each from both the sexes, one each case of mesothelioma, ovary & breast carcinoma in female and one case of colon carcinoma in male. In remaining 6 cases (50%) primary could not be detected as patients were lost to follow up or expired. Expired means patient died before definite primary could be established. Similar findings were noted in Udasimath et al¹² and Shobha et al⁵.

In the present study by combined approach of conventional cytosmears and cellblock technique, diagnostic yield for malignancy was significantly increased by cellblock method. An additional of 6 cases of malignancy was identified by cellblock method out of 12 cases. 5 cases were given as suspicious of malignancy & 1 case as benign by conventional smears so additional yield was 50% in

cellblock method. Dekker and Bupp et al¹ study reported that samples obtained by cell block method and smear technique for malignant lesions were double that of conventional smear technique. By using cell block method, tumors were subsequently demonstrated in 38% of patients who had negative or atypical cytological reports. In a study by Khan et al⁹, obtained an additional yield of 20% greater than that obtained for specimen examined in conventional smears method. Similarly, in a study by Shobha et al⁵ and Saraswathi et al² obtained an additional yield of 46.15% and 24% respectively. A major disadvantage of the cellblock is more turnaround time as compared to conventional cytosmears.

Thus the study concluded that using the combined approach on the same specimen leads to a more accurate diagnosis. So a combined approach of conventional cytosmears and cellblock technique helps us to get an additional yield for malignancy. Cell block of pleural effusion is more useful in cases suspected of malignancy compared to inflammatory effusions.

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