



CYTO-DIAGNOSIS OF PLEURAL EFFUSIONS: A COMBINED APPROACH TO MORPHOLOGICAL FEATURES IN ROUTINELY STAINED CYTOLOGY SMEARS AND CELL BLOCK TECHNIQUE

Pathology

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ABSTRACT

Introduction: Pleural cavity is a potential space between the parietal pleura and visceral pleura. It consists of some amount of fluid called pleural fluid which is normally less than 25ml. Pleural fluid is produced by parietal lining and absorbed by visceral lining. Fluid is produced by plasma filtration through capillary endothelial cells.

Aims & Objectives: A combined approach of cytology and cell block technique in diagnosing the cause of pleural effusion. To evaluate the utility of cell block preparation over the conventional smear in the cytodiagnosis of serous effusions.

Material & Methods: The present study was conducted on patients having pleural effusions. The cases and specimens were selected from the outdoor as well as indoor patients admitted in a tertiary care centre of North India and also from those sent directly to Pathology department from other hospitals. The duration of study was from January 2019 to September 2020.

The specimens obtained were processed in the biochemistry, cytopathology and histopathology laboratory in the Pathology department of GSVM Medical College, Kanpur.

Result And Analysis: Out of 100 cases studied it was found that 83 (83%) cases were of reactive pleural effusion and 17 (17%) cases were of malignant pleural effusions. Out of 17 malignant effusion, 16 (94%) cases were exudative, while 1 (6%) case was transudative. Only 8 cases of adenocarcinoma were diagnosed on smear examination, while 11 cases were diagnosed on cell block examination. 3 cases of poorly differentiated carcinoma were diagnosed on conventional smear and 5 cases on cell block examination. Out of total 17 malignant pleural effusions diagnosed by cell block, only 12 cases were diagnosed by conventional smear examination. Hence diagnostic yield increased by 30% using cell block preparations.

Conclusion: Thus it was concluded that routine centrifuge is not satisfactory in reporting fluids with scant cellularity. Hence for fluids with scant cellularity cell block preparation is a useful method. Also the morphology of the cells were well appreciated by cell block as compared to routine centrifuge, thus aiding in accurate diagnosis. In this study the diagnoses which were missed or incompletely diagnosed on conventional smear were diagnosed by cell block.

KEYWORDS

Cyto-diagnosis, Pleural Effusions, Morphological Features, Stained Cytology Smears And Cell Block Technique

INTRODUCTION

Pleural cavity is a potential space between the parietal pleura and visceral pleural.¹

These layers are separated by a thin layer of lubricating fluid that facilitates the movement of two membranes against each other. Pleural fluid is normally less than 25ml. and produced by plasma filtration through capillary endothelial cells. The accumulation of fluid in a body cavity is referred as an effusion. All effusions are pathologic regardless of their cellular constituents.²

Aspiration of serous cavities is a simple and relatively less invasive technique to achieve a diagnosis. Cytological examination of serous fluid is of paramount importance, it reveals information about inflammatory conditions of serous membrane, parasitic infestation, infection with bacteria, fungi, viruses, malignancy and also supply evidence of fistulous connection with a serous cavity. Thus first it assists the clinician in order of priority a list of differential diagnosis to follow. Second it allows the clinician to follow the result of therapy. Repeated aspiration of fluid may also be indicated by an unexplained poor response to therapy and may identify secondary complication which must be treated. Cytological examination not only helps for diagnosis of cancer but also 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.³

The diagnostic performance of cytological study of fluid may be attributable to the fact that cell population present in sediment is representative of larger surface area than that obtained by needle biopsy.

Cell block preparation is useful in examination of effusion. Cell block when combined with smears may improve the accuracy of the test by demonstrating the architectural pattern of cell aggregates and by facilitating the performance of histochemical and immunocytochemical stain.⁴

AIMS & OBJECTIVES

- A combined approach of cytology and cell block technique in diagnosing the cause of pleural effusion.
- To evaluate the utility of cell block preparation over the conventional smear in the cytodiagnosis of serous effusions.

MATERIAL & METHODS

The present study was conducted on patients having pleural effusions. The cases and specimens were selected from the outdoor as well as indoor patients admitted in a tertiary care centre of North India and also from those sent directly to Pathology department from other hospitals. The duration of study was from January 2019 to September 2020.

The specimens obtained were processed in the biochemistry, cytopathology and histopathology laboratory in the Pathology department of GSVM Medical College, Kanpur.

The following predetermined working proforma was used in the present study.

WORKING PROFORMA

Clinical Evaluation

History

Patients particulars, including name age gender socioeconomic status, genetic factors, environmental factors, dietary habits, smoking and clinical history of each patient was taken with special reference to the following points:-

- History of present illness
- History of past illness
- Family history

Examination Of Fluid:

General

1. Volume
2. Appearance

3. Coagulum
4. Odour

Biochemical Examination

1. Protein
2. Sugar
3. Cell count

Cytological Examination

Histopathological Examination Of Cell Blocks

Other Investigations - like x-ray, CT scan, ultrasonography.

Sampling Technique:

Pleural fluid was collected by the referring clinicians in a clean dry container, and brought to the pathology department as soon as possible.

Sometimes due to inevitable reasons, when the specimens were not brought immediately to the laboratory, then they were stored in refrigerator at 4°C. These specimens were brought to the room temperature before carrying out the procedure.

RESULT AND ANALYSIS

Our study showed that out of 100 cases studied it was found that 83 (83%) cases were of reactive pleural effusion and 17 (17) cases were of malignant pleural effusions. The non malignant cases were comprised of Tuberculosis 37 cases (44.6%), pneumonia 16 cases (19.3%) congestive heart failure 13 cases (15.7%), Liver cirrhosis 8 cases (9.6%) and 9 cases (10.8%) of other infections. On analyzing the age and sex distribution, it was found that out of 100 cases, 62 (61.0%) were male and 38 (39.0%) cases were female. M : F ratio 1.6:1 showing higher incidence of effusions in males. Out of total cases, 13 (19.5%) were reported in first three decades whereas the percentage increased to 80.5% with a total of 82 cases in later decades. Out of total 83 cases of reactive effusion, 62 (75%) were exudative while 21 (25%) cases were transudative, based upon their protein content. Out of 83 cases of reactive effusions, 56 (66.0%) cases had sugar content more than 60mg/dl while 27 (34.0%) cases showed values less than 60mg/dl. Out of total 21 cases of transudative effusion, 13 (76.4%) cases showed <500 cells/cumm, 04 (23.6%) cases had cellularity ranging from 500-1000 cells/cumm and none had cellularity more than 1000 cells/cumm. We found that in out of 21 cases of transudative effusion, 18 (85.72%) cases presented with predominance of lymphocytes and 3 (14.28%) cases showed predominance of mesothelial cells. Out of 62 cases of exudative effusion, only 06 (7.8%) cases showed <500 cells/cumm, 16 (33.3%) cases had cellularity ranging from 500-1000 cells/cumm and 40 (58.9%) cases had cellularity > 1000 cells/cumm. Out of 62 cases of exudative effusion, 37 (59.68%) cases presented with predominance of lymphocytes, 13 (20.97%) cases showed predominance of mesothelial cells, while 12 (19.35%) cases showed predominance of neutrophils. Out of 17 malignant pleural effusion 9 cases (53%) were females and 8 cases (47%) were males. Female : Male ratio is 1.12:1. Out of 17 malignant effusions, 10 (42.0%) cases had sugar <60 mg/dl while 7 (59%) cases had sugar >60 mg/dl. Out of 17 malignant effusions, 16 (94%) cases were exudative, while 1 (6%) cases was transudative. Only 8 cases of adenocarcinoma were diagnosed on smear examination, while 11 cases were diagnosed on cell block examination. 3 cases of poorly differentiated carcinoma were diagnosed on conventional smear and 5 cases on cell block examination. Out of total 17 malignant pleural effusions diagnosed by cell block, only 12 cases were diagnosed by conventional smear examination. Hence diagnostic yield increased by 30% using cell block preparations.

Table: Clinical Diagnosis, Protein Content (gm/ dl) And Predominant Cell Of Reactive Pleural Effusion Samples Received

		No. of cases	% of cases
Clinical diagnosis	Tuberculosis	37	44.6
	Pneumonia	16	19.3
	CHF	13	15.7
	Liver cirrhosis	8	9.6
	Other infections	9	10.8
	Total	83	100
Protein content (gm/ dl)	> 3 (exudative)	62	74.69%
	< 3 (transudative)	21	25.31%
	Total	83	100

Predominant cell	Lymphocytes	18	85.72
	Mesothelial cells	3	14.28
	Neutrophils	00	00
	Total	21	100

Table: Distribution Of Malignant Pleural Fluid Cases On The Basis Of Conventional Smear And Cell Block Examination

Type of malignancy	Smear	Cell block
Adenocarcinoma	08	11
Poorly differentiated carcinoma	03	05
Mesothelioma	01	01
Total	12	17

DISCUSSION

Reactive mesothelial cells, abundance of inflammatory cells, and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional smear. For these reason in this study an attempt is made to prepare and analyze both smears and cellblock. In this study, 10% alcohol-formalin was used as a fixative for cellblock preparation.

By this better cellularity was obtained compared to conventional smears as formalin minimized the cell loss by forming protein cross links. Similar fixative was used in study by Bodele et al (2003).⁵

In the present study, we found that out of 100 cases reactive effusions comprised of 83% cases and 17% malignant effusions. The non malignant cases comprised of Tuberculosis 37 cases (44.6%), pneumonia 16 cases (19.3%) congestive heart failure 13 cases (15.7%), Liver cirrhosis 8 cases (9.6%) and 9 cases (10.8%) of other infections.

This study is in agreement with Meenu Thapar et al (2009).⁶

This study is not in agreement with study done by Luse and Reagan in 1954.⁷ According to them the majority of cases were due to underlying CHF comprising of 18% of cases followed by cirrhosis and TB comprising of 11 & 8% of cases respectively. The reason for this discrepancy could be due to the fact that high prevalence of Tubercular infection is seen in India especially Uttar Pradesh.

It was found that on analysing the age and sex distribution, it was found that out of 100 cases; 62 (62.0%) were male and 38 (38.0%) cases were female with Male : female ratio of 1.6:1. Out of the total cases 17 (17.0%) were reported in 1st 3 decades, whereas the % increased to 83.0% with a total of 83 cases in later decades. In this study reason for high incidence of pleural effusion is seen in males due to high prevalence of smoking of Tobacco and consumption of alcohol as compared to females.

This study is in agreement with Adeoye et al (2017).⁸

Khan et al (2007)⁹ studied on pleural effusions in 22 patients. On demographic analysis high proportion of patients were male (77.28%) and females comprised only 22.72%, with a male : female ratio of 3.4:1. This could be due to small sample size studied by the authors.

Our study showed that out of total 83 cases of reactive effusion, 74.69% were exudative while only 25.31% cases were transudative.

The present study is in accordance with the study of James et al (2010)¹⁰ who studied 48 cases of pleural effusion and found that 45 (93.7%) cases were exudative and 3 (6.3%) cases were transudative.

In the present study, out of total 21 cases of transudative effusions, 16 (76.19%) cases showed <500 cells/cumm, 05 (23.81%) cases had cellularity ranging from 500-1000 cells/cumm. 85.72% cases showed predominance of lymphocytes. Out of 62 cases of exudative effusions, 6 (7.8%) cases showed <500 cells/ cumm, 16 (33.3%) cases had cellularity ranging from 500-1000 cells/ cumm and 40 (58.9%) cases had cellularity >1000 cells cumm. 59.68% cases of exudative effusions showed predominance of lymphocytes and 19.35% cases showed predominance of neutrophils.

The present study is in concordance with Swamy et al (2010)¹¹ who studied 200 cases of pleural effusion, and found that out of 40 cases of transudative effusions, 75% cases had cellularity ranging from 0-500 cells cumm. and remaining 25% cases had cellularity between 500-

1000 cells/cumm. 80% samples of transudative effusions had more than 50% lymphocytes. Among 160 cases of exudative effusions 7% cases showed <500cells/cumm, 43% cases had cellularity ranging from 500-1000 cells/ cumm. 63% cases presented with predominance of lymphocytes, while predominantly neutrophils were seen in 31% cases.

In our study female predominance in case of malignant pleural effusion with female : male ratio of 1.12:1. Thus this study is in agreement with study done by Sears et al in 1987.¹² He found female to male ratio of (1.55:1) in malignant pleural effusion. Out of 17 malignant effusions, 16 (95.8%) were exudative, while 1 case was transudative. Transudative effusion in malignancy may be because the case had concomitant anemia and hypoproteinemia.

This is in concordance with the study done by Kushwaha et al (2008)¹³ on pleural effusions. They studied 28 malignant effusions and found that 88% of malignant effusions were exudative except few cases were transudative.

We also found that in present study, among the malignant pleural effusions, out of 11 cases of adenocarcinoma, only 8 were diagnosed on smear examination. This was because 3 cases were missed due to low cellularity. On subsequent cell block examination, low cellularity cases in conventional smear showed enough clusters of atypical cell forming acini to classify as adenocarcinoma. 3 cases diagnosed as poorly differentiated carcinoma on routine centrifuge smear and 3 cases were missed due to scant cellularity and marked distortion in the nuclear and cytological details. These 3 cases were positive on cell block preparation due to enhanced cell details and increased cellularity in cell block.

In the present study, out of 17 cases of malignant effusions, 12 were diagnosed by conventional smear, 17 were diagnosed on cell block examination. So the diagnostic yield increased by 30% on cell block examination.

This is in concordance with study done by Dekker et al (1978),¹⁴ they reported the samples obtained by mini cell block technique and conventional smear technique of malignant peritoneal effusions and concluded that on cell block examination additional diagnostic yield was 38%.

The use of cell block not only increases the cellularity as compared to conventional smear but also the cells were evenly distributed. The cellular morphology, nuclear and cytoplasmic details, were better appreciated on cell block. Also cell block carries additional advantage of performing Immunohistochemistry which aids in the diagnosis and can be used for retrospective analysis.

Reactive mesothelial cells have been responsible for simulating malignancy in conventional smear, largely due to formation of rosette, pseudoacini or acini, with or without the presence of prominent nucleoli. The cell block effectively puts both the features in their proper perspective: That is nucleoli do not appear as prominent as in conventional smear, and the pseudoacinar and acinar pattern can be better appreciated when, in the cell block. The other advantage of cell block is the concentration of cellular material in one small area that can be evaluated at a glance with all cells lying in the same focal plane of the microscope. It bridges the gap between cytology and histology. Also, the cellularity was more by cell block method as compared to routine centrifuge method. Architectural pattern such as glands, sheets, clusters and cell balls were commonly observed in cell block method.

CONCLUSION

1. Majority of cell block were diagnostically superior as compared to conventional smears.
2. The male: female ratio was 1.62:1.
3. Majority of cases were reported in patients more than 30 years of age (80.5%).
4. Most of the pleural effusions were exudative (75%)
5. Majority of the reactive effusions had sugar more than 60mg/dl (66.1%).
6. Most of the transudative effusions showed less than 500 cells/cumm (76.4%) and maximum cases presented with predominance of lymphocytes.(88.0%)
7. Most of the exudative effusions had cellularity >1000 cells/cumm (58.9%) with majority showing predominance of lymphocytes.

8. Most of the malignant effusions were exudative (95.8%)
9. Most malignant effusions had normal sugar content.
10. Out of 17 malignant pleural effusions diagnosed on cell block only 12 were diagnosed on conventional smear examination.
11. Thus using cell block methods diagnostic yield is increased by 30%.

Thus it was concluded that routine centrifuge is not satisfactory in reporting fluids with scant cellularity. Hence for fluids with scant cellularity cell block preparation is a useful method. Also the morphology of the cells were well appreciated by cell block as compared to routine centrifuge, thus aiding in accurate diagnosis. In this study the diagnoses which were missed or incompletely diagnosed on conventional smear were diagnosed by cell block.

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