Study of pleural fluid cytology in a tertiary care hospital

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Abstract

Introduction: Identification of malignant cells in any body fluid always poses a challenge for any cytopathologist. This often requires additional information like clinical history, morphological evaluation and sometimes modern techniques like cytochemistry and immunohistochemistry. This will help the clinician, surgeon and oncologist in treating the patient and it is the determining factor for treating patient.

Materials and Methods: It is an observational, prospective study. The data was collected from the patients who were admitted in our hospital which is situated in Ahmedabad. Total 100 cases of pleural fluid cytology were studied over 2 years period and analysed.

Result: We studied 100 cases of pleural effusion cytology and we found that out of 100 cases, 83 cases were of non malignant effusion and 17 cases were of malignant effusion.

Conclusion: Definite diagnosis of pleural fluid effusion can be done by doing cytology in most of the cases, however in some cases adjuvant techniques such as cytochemistry, immunohistochemistry, ploidy and proliferation markers are found very handy. Pleural fluid cytology is the gold standard and still the first line of investigation in ruling out neoplastic lesions. It is also useful in sub typing of non neoplastic lesions and helps clinician in management of patients.

Keywords: Pleural fluid, Cytology, Malignant cells.

Introduction

Cytological study of body fluids has a long way in the history of pathology as it is an inexpensive, simple procedure to perform. There are three serosal body cavities in our body comprising of pleural, peritoneal and pericardial cavity.[1] These cavities are lined by parietal and visceral layer of epithelium. Normally they contain very little amount of fluid which is required for lubricating underlying viscera. Accumulation of fluid is known as effusion which occurs due to imbalance between fluid formation and removal.[2] Pleural fluid effusion is one of the most common effusions which encountered in day to day medical practice. Pleural cavity is one of the three serous cavities of our body; each cavity is made up of a double layered serous membrane namely visceral layer and parietal layer. Pleural cavity contains only a small volume of lubricant fluid. Disturbances of the mechanism that normally maintains the dynamic flow nay result in accumulation of excess fluid. Two types of effusion are recognized, transudates & exudates. Transudate is generally thin and watery contains very few cells and low protein. Exudate is generally thick and viscous and rich in cells and proteins. Other type of effusion is neoplastic and chylous.[3]

Cytology of pleural fluid is the low cost, rapid and safe investigation and of valuable investigation for diagnosis of cancer and for staging and prognosis of the patients. Apart from help in cancer detection, it also helps regarding systemic pathology and various inflammatory conditions of the pleural cavity.

The study of pleural fluid cytology has paramount importance in identifying atypical cells in effusions which in turn helps to know the advancement of the disease process in the body.[4]

The role of pathologist in malignant pleural fluid effusion is to identify cancer cells accurately and to identify the tumor type and if possible the site of primary origin. The most common type of tumor to produce metastasis in pleural cavity is the broad group of adenocarcinomas, most often from Lung and also common are from breast, GIT. However there are occasions when unusual malignancies are encountered. The diagnosis of such requires a constellation of cytomorphological criteria and correlation with the clinical history and other investigation of the patients.

The rate of diagnostically equivocal effusions in routine cytology is dependent on the effusion examined, type of preparation and staining, experience of examiner, clinical history and application of ancillary method.

Objectives

1. To study the pleural fluid effusion for the diagnosis of inflammatory, neoplastic, infective or immune mediated lesions.
2. To assess the proportion of neoplastic and non - neoplastic condition causing pleural fluid effusion.
3. As a diagnostic tool for typing of tumor in malignant pleural effusions.
4. To help surgeons and oncologists in the staging and further management of the patients.

Materials and Methods

It is observational, prospective study of 2 years carried out at a tertiary care hospital. Thoracocentesis or pleural tap is an invasive procedure to remove fluid from pleural cavity and performed by clinician. We received 100 cases of pleural effusion for cytology at our department along with pretested proforma, which include clinical findings, clinical diagnosis & other supportive investigations. Sample was

received in sterile plastic container. Fresh sample of pleural fluid was evaluated for the study. From the received fresh sample, 5 ml fluid was taken and fluid was centrifuged at 2500 rpm for 15 minutes and a minimum of four thin smears were prepared from the sediment and were immediately fixed in 95 % alcohol and stained with haematoxylin & Eosin stain. Other stains like Giemsa stain was used whenever required. After confirming final diagnosis, each data was analysed. Samples which were less than 5 ml, not received in sterile container were excluded from our study.

Results

Total 100 cases of pleural fluid effusion were received and studied.

Table 1: Distribution of the sample by age and sex.

Age groups (In Years)	Sex				Total	
	Male		Female			
	No	%	No	%	No	%
0-10	00	0.00	00	0.00	00	0.00
11-20	02	3.63	04	8.88	06	6.00
21-30	07	12.72	06	13.33	13	13.00
31-40	06	10.90	13	28.88	19	19.00
41-50	16	29.09	10	22.22	26	26.00
51-60	14	25.45	07	15.55	21	21.00
61-70	07	12.72	04	8.88	11	11.00
71-80	02	3.63	01	2.22	03	3.00
81-90	01	1.81	00	0.00	01	1.00
Total	55	100.0	45	100.0	100	100.00

Maximum number of patients was in the age group of 41-50 years accounting 26 % of total cases. In male, maximum number of cases was in the age group of 41-50 while in female it is 31-40 years. Male to Female ratio is 1.22:1.

Table 2: Cytodiagnosis of pleural fluid Effusion.

Sex	Non- Neoplastic		Neoplastic		Total	
	No	%	No	%	No	%
Male	43	51.8	12	70.5	55	55.0
Female	40	48.2	05	29.5	45	45.0
	83		17		100	100.0

Out of 100 cases, 83 % cases were of non neoplastic lesion while 17% cases were of neoplastic lesion. Neoplastic lesion had higher male female ratio 2.4:1 than neoplastic effusion which is 1.07:1.

Table 3: Distribution of Non - Neoplastic Effusion.

Distribution of Non Neoplastic Effusion	No	%
Changes Acute Inflammation	15	18
Changes of Chronic Inflammation	68	82
Total	83	100.00

Among non neoplastic effusion maximum cases were of chronic inflammation accounting 82 % followed by changes of acute inflammation were associated in 18% cases. Out of 68 cases of chronic inflammation 12 cases were suggestive of tuberculous inflammation characterised by lymphocytes rich effusion.

Table 4: Distribution of Neoplastic effusion with primary identified

Primary organ	No	%
Lung	05	29.40
Breast	03	17.64
GIT	02	11.76
NHL	01	5.88
Malignant Mesothelioma	01	5.88
Unknown	05	29.40
Total	17	100.00

It was observed that lung was the most common site of primary tumor accounting 5 cases, followed by Breast with 3 cases, GIT with 2 cases, Non Hodgkin's Lymphoma and malignant mesothelioma each with 1 case. Primary site of tumor was not detected in 5 cases where patients were lost in follow up.

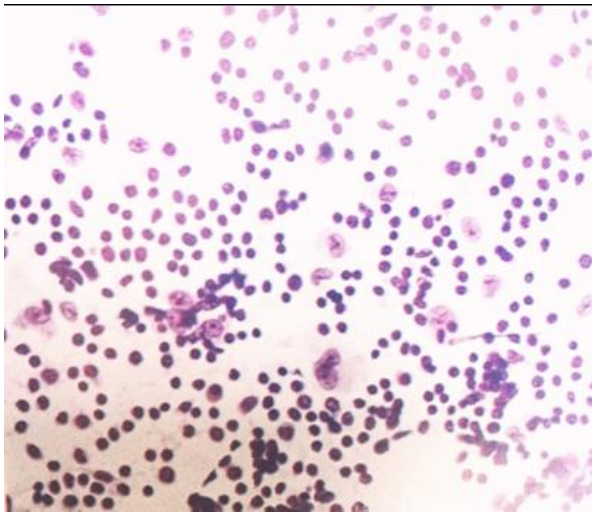

Fig. 1: Lymphocyte rich effusion (H & E stain) 10x

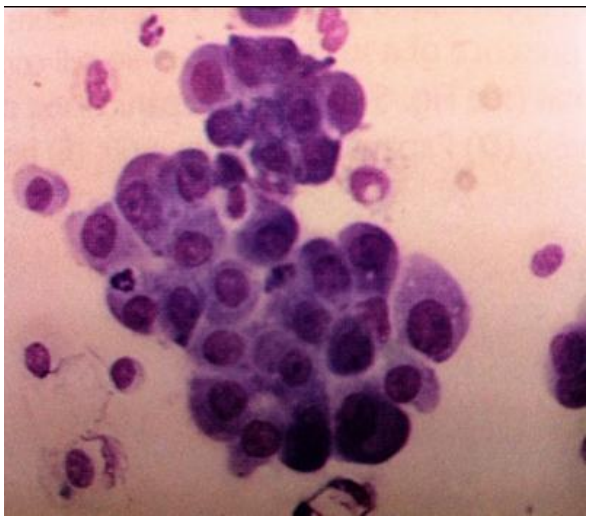

Fig. 2: Malignant Mesothelioma(Giemsa stain) 40x

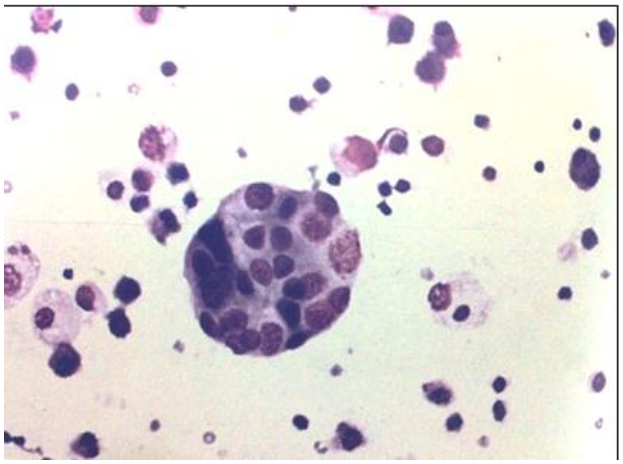

Fig. 3: Cannonball formation in Breast

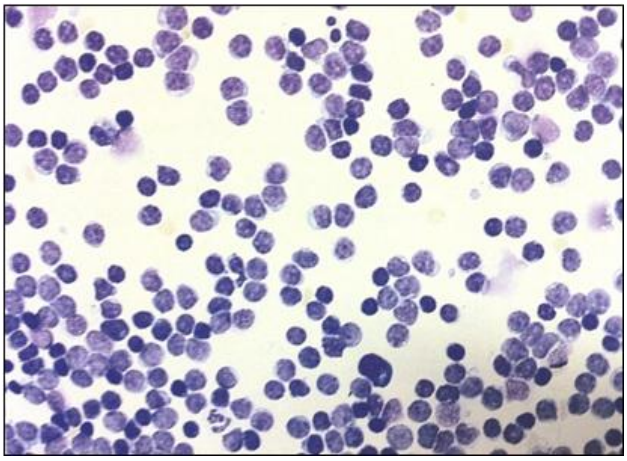

Fig. 4: Non Hodgkin's Lymphoma Carcinoma (H & E stain)

Discussion

Diagnostic cytology is the scientific art of interpretation of cells from the human body that exfoliate or are removed from their physiologic millieu. Cytodiagnosis of pleural fluid represents the cell population from a much larger representative area than that obtained from needle biopsy.[5] Cytology has a greater opportunity than needle biopsy technique to retrieve malignant cells. In the present study of 100 cases of pleural effusion, Non neoplastic effusions were

accounting for 83 cases while neoplastic effusion were accounting for 17 cases.

In non neoplastic effusion, various inflammatory cells like lymphocytes, neutrophils, plasma cells were found. Out of 83 cases, highest numbers of cases were from mixed inflammatory cells accounting for 51 cases, followed by predominant lymphocyte rich effusion accounting for 12 cases, predominant mesothelial cell effusion accounting for 18 cases and 2 cases are from predominant neutrophil rich effusion.

Our study is comparable with study by Epstein *et al.*[6] and stated that the majority of tuberculous effusions had more than 50% lymphocytes, 90% cases had had greater than 5 gm/dl of protein and 85% cases had glucose greater than 50 gm/dl. In our study tuberculous effusion rarely had mesothelial cells more than 5 % which are in line with that of study by Aggarwal *et al.*[7]

All pleural fluid were tested for pH, glucose, proteins, LDH, total ADA, microscopy and microbial testing (Grams staining, Z N Staining, cultures). Pleural fluid Adenosine Deaminase (ADA) nowadays widely used for confirmation of tuberculosis inflammation in case of lymphocyte rich effusion while fluid protein and fluid LDH are used for diagnosis of exudates.

Cases with mixed inflammatory cells were of pneumonia, hypoproteinemia and non specific inflammation. 12 cases of lymphocytes rich effusion were correlated clinically with history of tuberculosis.

We found 18 cases those had mesothelial cells as predominant cells. Mesothelial cells are round and have a single central or eccentric nucleus. Some clusters of mesothelial cells show clefts or "windows" in between them. Characteristic feature of mesothelial cells described by Koss is the flattening of the opposite cell membrane with the formation of clear gaps or "windows" between two cells. It is because of microvilli separating these cells. Bedrossian insists that in benign mesothelial cells these microvilli are slender, bushy and distributed evenly around the cells whereas in adenocarcinoma, if present they are concentrated at the poles and are short and stubby. These mesothelial cells due to their appearance sometimes look like malignant cells.

Malignant cells have moderate cytoplasm, hyperchromatic, pleomorphic nuclei, granularity of the chromatin and abnormal mitoses with prominent nucleoli and form gland like or tubular structures with central lumina also referred by some as spheroids or hollow sphere. 3 dimensional clusters and complex papillary clusters are also seen.

A single variable which strongly predicts malignancy is "bloody fluid". In our study 68.97% of hemorrhagic effusions were positive for malignancy. But all hemorrhagic fluids need not be due to malignancy and non-hemorrhagic fluids can have malignant cells.[8]

Irregular nuclear membranes, nuclear moulding with absence of "windows", are the features which are useful to differentiate them from mesothelial cells.

Cytospin and cell block techniques are extremely useful in improving cell yield of pleural fluid effusions and ensure high diagnostic efficacy especially when cellularity is low. They also have advantage of better preservation of cellular morphology compare to conventional method.

In our study, among the malignant lesions diagnosed in analysis of 17 cases of pleural fluid effusion, primary site of tumor is more common in lung (29%), followed by Breast (18%), GIT (12%), NHL and Malignant Mesothelioma each accounting (6 %). Our study correlate with other studies as per see table V being lung is the most common primary site of tumor. We found 17 cases of neoplastic effusion, with male to female ratio of 2.4:1.

Table 5: Pleural fluid analysis for primary lesions in various studies

S. No	Study	Breast %	Lung %	Ovary %	GIT %	Others %	Unknown %
1	Lopez & Cardoz[9]	16	21	4	3	07	49
2	Johnston[10]	15	36	8	6	16	19
3	Khan et al[11]	12	69	0	0	0	19
4	Present study	18	29	0	12	12	29

In some difficult cases new techniques like immunohistochemistry can help in final diagnosis. In general, the best positive mesothelioma tissue markers are Calretinin, CK 5/6, WT1 and podoplanin. CEA, MOC-31, B72.3 and Ber- EP4 are the best negative markers to distinguish between epithelioid mesotheliomas and adenocarcinomas. Monoclonal antibody D2-40 has been proved to be helpful in distinguishing between epithelioid mesotheliomas and adenocarcinomas nowadays.

Conclusion

Confirmative diagnosis of pleural fluid effusion can be achieved by cytological analysis in most of the cases, however in some cases novel techniques such as cytochemistry, immunohistochemistry, ploidy and proliferation markers are being found very handy. Pleural fluid cytology is a very cost effective first line of investigation and important to clinician, surgeon for early diagnosis, staging, and prognosis of disease and helpful in management of patients.

Conflicts of Interest: None.

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