



CYTOMORPHOLOGICAL STUDY OF BODY FLUIDS: A TERTIARY CENTER STUDY

Dr Khushboo Kumari

Junior Resident (academic) Deptt Of Pathology RIMS Ranchi.

Dr Manoj Kumar Paswan

Associate Professor, Deptt Of Pathology RIMS, Ranchi.

Dr Vinay Bhushan*

MBBS MD(Pediatrics), Ex-Senior Resident UCMS& GTBH(New Delhi), Specialist Pediatrician at SBMCH Hazaribagh Jharkhand (India).*Corresponding Author

ABSTRACT

BACKGROUND: Body fluids like pleural fluid, peritoneal fluids normally present in the body with their constituents in particular proportions in minimal quantities, within serous body cavities. These fluids undergo abnormal qualitative and quantitative changes in various pathological conditions **AIM:**(i) Cytological examination of pleural, peritoneal fluid received in the department of pathology, RIMS Ranchi. (ii) An attempt to establish the cause of the effusion in pleural and peritoneal fluid (iii) To establish etiological causes of pleural effusion and ascites with respect to age group distribution.(iv)To draw the necessary conclusions with statistical analysis. **MATERIAL AND METHODS:** The smears were prepared using conventional centrifugation techniques & stained with hematoxylin & eosin/Papanicolaou stains. Then the smear were studied to see cell morphology & pattern.**RESULTS:** Out of 40 samples of pleural & ascitic fluids, majority of the cases belonged to predominantly lymphocytes pattern. And 5/40 in pleural fluid, 4/40 in ascitic fluids were carcinomas. **CONCLUSION:** The study of exfoliated cells in fluid cytology can be used as a routine diagnostic procedure and the techniques used for processing fluids are easy to perform in any laboratory set up. The technique like conventional centrifugation help in better morphological interpretation; therefore the need to reconfirm its utility in diagnostic cytology.

KEYWORDS : Pleural fluid, peritoneal fluid, Cytology, Centrifugation, Haematoxylin & Eosin, Papanicolaou.

INTRODUCTION

Exfoliative cytology is the study of spontaneously shed cells lining an organ or a cavity, from where they are removed by non-abrasive means^{1a}. It included study of cells from anatomic areas like effusion, CSF and synovial fluids as well as cells shed from urinary, respiratory and female genital tracts.

The principle features of exfoliative cytology are^{1b}:

- The technique is applicable to organs with easy access from where the samples can be obtained.
- The samples often contain a great variety of cells of various types from many different sources-inflammatory cells, macrophages, microorganisms, and material of extraneous origin.
- The cellular constituents are sometimes poorly preserved due to ongoing exfoliation from time to time.
- The single most advantages of exfoliative cytology is the facility with which multiple samples can be obtained from the same site.

The diagnosis of cancer in pleural, pericardial or peritoneal fluids is of paramount importance for the patient and the attending physician or surgeon^{1c}.

In the respiratory tract non-malignant and malignant causes of effusion can be identified by the relatively non-invasive technique of pleural fluid cytology. The cell population present in the sediment is representative of a much larger surface area than that obtained by needle biopsy. Cytologic study of pleural fluid is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as, in certain cases, a means of prognostication of disease process.² Ascitic fluid examination provides a valuable clue to the etiological diagnosis of ascites particularly in cases where the clinical picture is not straight forward.³ Among many causes of ascites, decompensation of chronic hepatic cirrhosis accounts for 80% of the cases, followed by tumors which account for 10% of cases, congestive heart failure and inflammatory conditions account for 3% of cases each; other causes such as nephritic syndrome, exudative enteropathy and chylous ascites are rare⁴. Short of a peritoneal biopsy, bacteriological and cytological examination of ascitic fluid is the only procedure to confirm the diagnosis of TB and malignancy. Peritoneal fluid washing are useful for predicting the prognosis of gynecological, gastric, pancreatic and colorectal malignancies.⁵ Pericardiocentesis and fluid cytology is a standard diagnostic tool in the evaluation of pericardial effusion. Malignant involvement of pericardium and heart is found in from 0.1 to 6.4 % of unsuspected autopsies and in up to 20.6% of patients dying of cancer. Pericardial fluid cytology is accurate and positive test results are completely reliable⁵

The various methods involved in processing of fluids^{1d}:

- Direct centrifugation of fluid, pouring out the supernatant and making smears on glass slides with the cell button.
- Cytospin preparations
- Preparation with membrane filters
- Cell block method

Different staining methods have been used in preparation including Romanowsky stains, Papanicolaou stain, hematoxylin-eosin and toluidine blue.

MATERIALS AND METHODS**Source of data:**

Cytology samples obtained from department of pathology, RIMS Ranchi, which were came for cytological examination –Samples came from all departments of RIMS Ranchi.

For all samples case detailed i.e. clinical information with regards to age, sex, history, provisional diagnosis etc was taken.

Sample fixation:

- Pleural and pericardial fluids were collected after pleural and abdominal tap.
- Fresh samples were processed soon after collected. Where there was a delay in processing, the samples were fixed using absolute alcohol (1 part of fluid:10 part of alcohol)

Methods of fluid processing:**Conventional fluid processing by centrifugation:**

- Around 5ml of sample was taken and placed in a test tube for centrifugation.
- It is centrifuged at 3000rpm for 10 min.
- The supernatant fluid was pipette out and the cell button was smeared, stained with haematoxylin and eosin/papanicolaou stains.

RESULTS**The following observations were made in this study on the body fluids.**

- Total no of cases subjected to cytological examination-80
- Distribution of cases:

Table no:1

PLEURAL FLUID	ASCITIC FLUID
40	40

- Out of the 80 samples, 55(68.75%) were from males and 25(31.25%) were from females. Male to Female ratio was 2.2, showing

a male preponderance.

The distribution of samples among males was as follows:25 of pleural fluid,30 of ascitic fluid.

The distribution of samples was among females was as follows:13 of pleural,12 of ascitic fluid.

iv.Age: The age distribution was

Table no: 2

Age category	No of cases observed	Percentage
0-20 yrs	17	21.25%
20-40 yrs	38	47.5%
>40 yrs	28	35%

V.Age and sex distribution with regard to specific anatomical sample:

Detail on pleural fluid: 40 cases

Table no:3

Age	Males	Females	Total
0-20 yrs	2	4	6
20-40 yrs	8	3	11
>40 yrs	15	8	23
Total	25	15	40

Detail on ascetic fluid: 40 cases

Table no: 4

Age	Males	Females	Total
0-20 yrs	1	2	3
20-40 yrs	12	4	16
>40 yrs	14	7	21
Total	27	13	40

Vi.Distribution of cases with regard to malignant and non malignant cases:

Table no: 5

	Malignant	Non-malignant
Pleural fluid	5	35
Ascitic fluid	4	36

In each category-

1. Pleural fluid: Out of the 40 cases, 5 were malignant. All were adenocarcinoma

2Ascitic fluid: Out of 40 cases, 4 were malignant.. All were adenocarcinoma

Vii. Following results were derived after examining conventional centrifuged smear:

A)PLEURAL FLUID: TOTAL NO OF CASES-40

Based on the predominant cell pattern, cases were categorized in to predominantly lymphocytes (21 cases), predominant polymorphs (9 cases), mixed cell population (3 cases), chiefly mesothelial cells (2 cases) and malignant cells (5 cases).

Table no:7

PREDOMINANT CYTOLOGICAL PATTERN	NO OF CASES	CYTOLOGICAL IMPRESSION
PREDOMINANTLY LYMPHOCYTES	21	CHRONIC INFLAMMATION
PREDOMINANTLY POLYMORPHS	9	ACUTE/SUPPURATIVE INFLAMMATION
MIXED	3	CHRONIC INFLAMMATION
POPULATION(LYMPHOCYTE S,POLYMORPHS AND MESOTHELIAL CELLS)		
CHIEFLY MESOTHELIAL CELLS	2	REACTIVE MESOTHELIAL CELL HYPERPLASIA
MALIGNANT CELLS	5	CARCINOMA

B)ASCITIC FLUID: TOTAL NO OF CASES -40

Based on the predominant cell pattern, cases were categorized in to predominantly lymphocytes (19 cases), predominant polymorphs (10 cases),mixed cell population (4 cases), chiefly mesothelial cells (3 cases) and malignant cells4 (cases).

Table no:8

PREDOMINANT CYTOLOGICAL PATTERN	NO OF CASES	CYTOLOGICAL IMPRESSION
PREDOMINANTLY LYMPHOCYTES	19	CHRONIC INFLAMMATION
PREDOMINANTLY POLYMORPHS	10	ACUTE/SUPPURATION INFLAMMATION
MIXED POPULATION	4	INFLAMMATION
CHIEFLY MESOTHELIAL CELLS	3	REACTIVE MESOTHELIAL HYPERPLASIA
MALIGNANCY	4	CARCINOMA

Etiologic causes of pleural effusion:

Pleural fluid: Out of the 40 cases studied, In the predominantly lymphocytes pattern, maximum no of cases 19 were proved to be tuberculosis .The diagnosis of tuberculosis was based on the clinical history, chest x-ray finding,ESR ,sputum examination and correlation with the cytology.Out of the 9 predominantly polymorphs pattern,6 cases were of pneumonia. Pneumonia cases were diagnosed with clinical history, chest x-ray finding, complete blood counts and cytological correlation. All the 5 cases of malignancy were adenocarcinomas.No mass lesion was detected on chest x-ray in these cases and these were diagnosed as metastatic adenocarcinoma.

In the 2 cases of predominantly lymphocytes pattern,3 cases of predominantly polymorphs pattern,3 cases of mixed cell pattern and 2 cases of chiefly mesothelial pattern, the exact etiology could not be ascertained. These cases were categorized in to other and patients were followed up for the treatment.

Table no:9

Age(years)	Tuberculosis	Pneumonia	Malignancy	Others
0-20 yrs	1	3	--	3
20-40 yrs	6	1	--	3
>40 yrs	12	2	5	6
TOTAL	19(47.5%)	6(15%)	5(12.5%)	10(25%)

Majority of the tuberculosis cases(12) and all the 5 malignancy cases were in the age group of above 40 years.

Etiologic causes of ascities:

Out of the 40 samples of ascitic fluids examined, In the predominantly lymphocytes pattern, majority of the cases (17) were due to cirrhosis/alcoholic liver diseases/chronic liver disease. The diagnosis was based on clinical picture, liver function tests, history of alcohol intake, ultrasound abdomen and cytology correlation. Two(2) of the cases of predominantly lymphocytes pattern and 2 cases of mixed cell population were proved to be due to tuberculosis. In these cases, the diagnosis was based on the clinical picture, history, ultrasonography of the abdomen and cytology correlation. Of the 10 cases of predominantly polymorphs pattern,1 cases were due to intestinal obstruction.

The diagnosis was based on clinical picture, ultrasound findings, cytology correlation and intraoperative findings where the surgery was done.3 cases were due to bacterial peritonitis, and diagnosis was based on the clinical history, predominantly polymorphs in the ascitic fluid and radiological.

In the malignant cell pattern, all the 4 cases were adenocarcinoma.

In 6 of the 10 cases of predominantly polymorphs pattern, 2 of the 4 cases of mixed cell pattern and all the 3 cases of mixed cell pattern ,the exact etiology could not be ascertained and these cases were categorized in to 'other'. The patients were followed up for the treatment.

Table no:10

AGE (years)	Cirrhosis/ alcoholic liver disease/ chronic liver disease	Tuberculosis	Intestinal obstruction/ bacterial peritonitis	Malignancy	Others
<20 yrs	1	-	1	--	2
20-40 yrs	7	2	1	1	3
>40 yrs	9	2	2	3	6
TOTAL	17	4	4	4	11

PHOTOGRAPHS

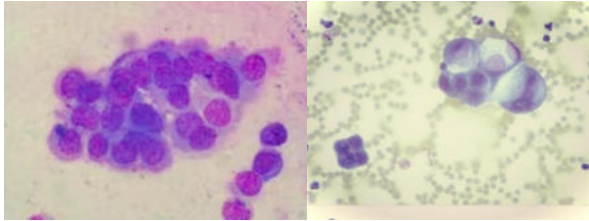


Fig 1: carcinoma cell in clusters in Fig 2: Carcinoma cell in ascitic fluid- conventional smear (100x)
ascitic fluid- conventional smear (100x)

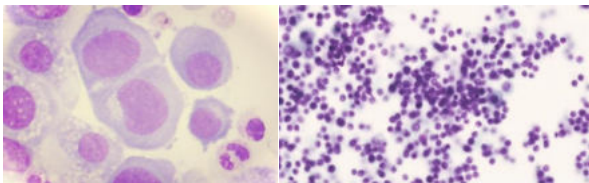


Fig 3: mesothelial cell in Fig 4: mixed cell population in ascitic fluid- conventional fluid-conventional smear (40x)
smear (100x)

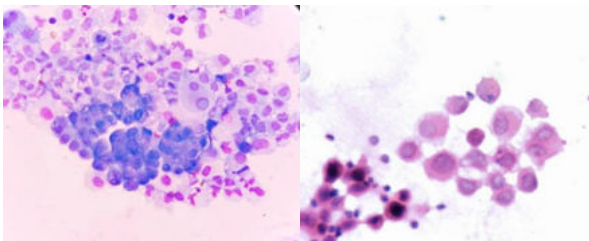


Fig 5: Malignant cells in pleural fluid- conventional smear (40x) Fig 6: Reactive mesothelial cell in pleural fluid-conventional smear (40x)

DISCUSSION

The body cavities in the human body are lined by the visceral and parietal layers of the mesothelium. The three important cavities include the pleural enclosing the lungs, the peritoneal enclosing gastrointestinal tract organs and pericardial surrounding the heart. In the absence of disease the parietal and visceral layers of these cavities are separated by a thin layer of lubricating fluid in order to facilitate the movements of the membrane against one another^{1c}

In different pathological condition these body cavities are filled with excess amount of fluid may be in form of either a transudate or exudates. Investigation of these effusion play role to rule out malignant and non-malignant conditions thus making the pathologist contribute positively to the clinical diagnosis and management of patient.

The overall sensitivity in the examination of pleural fluid has been reported in the literature to be in the range of 70% to 90%⁶ In the present study of the 40 samples of pleural studied malignancy could be diagnosis in 5 cases and was ruled out in 35 cases. Among the ascitic fluid samples 4 cases are diagnosed as malignant. Accuracy of ascitic fluid examination are lower as compared to pleural fluid ranging between 50% to 90% as reported by Junaid in 1980⁷.

Among the ascitic fluid samples 4 cases are diagnosed as being malignant. The rest suggested chronic inflammation with predominantly lymphocytes or reactive mesothelial hyperplasia. 19 cases of pleural fluid samples and 4 cases of ascitic fluid samples were diagnosis in the present series as being of tuberculosis etiology. Such a

finding of pleural effusion with predominance of lymphocytes in cases of tuberculosis was confirmed by logistic regression method by Ellison et al in 1998. As per the author, a few macrophages and mesothelial cells may be seen in these effusions. The present cytology confirmed these finding and in correlation with the clinical histories of the patient enabled a diagnosis of tuberculosis and excluded viral pneumonias and the remote possibility of lymphomas. In one case of pleural effusion, an early epithelioid granuloma was observed in the cells suggested a tuberculous etiology^{1f}.

CONCLUSION

Exfoliative cytology plays a helpful role in the diagnosis of neoplastic as well as inflammatory conditions associated with the pleural fluids and peritoneal fluids without surgical interventions. The procedure used is less invasive as compared to tissue biopsy and also help in the planning of treatment. The present study reconfirms and highlights its utility in diagnostic cytology.

ACKNOWLEDGEMENT

The author would like to thanks Dr vinay bhushan co-author for financial support, designing study and help in editing this manuscript. I would also like to thanks Dr Manoj Kumar paswan sir for his kind support in whole study procedure and thanks to staff of pathology department for their support for sample collection and staining.

Disclosure of conflict of interest: None

REFERENCES

1. Koss LG and Melamed MR (Eds). Koss' Diagnostic cytology and its histopathology bases. 5th edition. Philadelphia: JB Lippincott Company, 2006: pages : 11(a); 12(b); 950(c); 1576(d); 919(e); 933(f).
2. Kushwaha R, Shashikala P, Hiremath S, Basavaraj HG. Cells in pleural fluid and their value in differential diagnosis. Journal of cytology 2008; 25:138-43.
3. Kedarnath, Mittal H J, Mishra SD, Mohan A. Diagnostic value of ascitic fluid examination. JAPI 1968; 16:991.
4. Jha R, Shrestha HG, Sayami G, Pradhan SB. Study of effusion cytology in patients with simultaneous malignancy and ascites. Kathmandu University Medical Journal 2006; Vol 4(16):483-487.
5. Meyers DG and Bouska DJ. Diagnostic usefulness of pericardial cytology. Chest 1989; 95: 1142-1143.
6. Ehya H. Effusion cytology. Clinics in laboratory medicine 1991; Volume 11(2):443-467.
7. Junaid TA. Cytologic diagnosis of ascitic fluid in Ibadan, Nigeria. Journal of the national medical association 1980; 72(7):669-672.