

COMPARATIVE STUDY OF BODY FLUID CYTOLOGY USING CYTOSPIN-II AND ORDINARY CENTRIFUGEAshok Kumar Deshpande¹, Syamala Bhaskaran²**HOW TO CITE THIS ARTICLE:**

Ashok Kumar Deshpande, Syamala Bhaskaran. "Comparative Study of Body Fluid Cytology using Cytospin-II and Ordinary Centrifuge". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 40, May 18; Page: 6904-6910, DOI: 10.14260/jemds/2015/1003

ABSTRACT: INTRODUCTION: The cytologic examination of body fluids is a common practice and of distinct value in confirming or disapproving malignant metastatic tumors to the body cavities. It also gives information on specific pathologic processes as tuberculous or otherwise. **AIMS AND OBJECTIVES:** The present study was carried out to compare the sediments obtained by ordinary centrifuge and Cytospin II centrifuge using pleural and ascitic fluids as specimen samples. The emphasis was on cell yield and distribution, preservation of cell morphology, type of malignant cells and comparing the results for cases which were positive by both methods. **MATERIALS AND METHODS:** A total of 150 random samples (57 pleural and 93 peritoneal fluids) were studied over a period of one year and two months. The samples were split and processed by both methods simultaneously and results were compared. Histopathological correlation was done wherever possible. **RESULTS:** There were 25 cases (16%) of malignant effusions, 9(36%) from pleural and 16(64%) from peritoneal cavities. The most common primary site in female patients was ovary, whereas, in male patients it was the gastrointestinal tract. All 25 cases (100%) of malignancy were detected by Cytospin II method while the ordinary centrifuge picked up only 23 cases (92%). **CONCLUSIONS:** The comparative study between the Cytospin II and ordinary centrifuge revealed that the Cytospin II preparation gives a better yield of cells specially when they are scanty, morphology of cells is very well preserved and much time is saved in screening the slide. The Cytospin II is better in picking up malignant cells than the ordinary centrifuge.

KEYWORDS: Malignant effusions, Cytospin II, Ordinary centrifuge.

INTRODUCTION: The examination of effusions to determine the presence of malignant cells has been done for at least 130 years. Bahrenburg and Mandlebaum (1917),¹ before world-war II used the technique of cell block for effusion sediments. After world-war II, many began using the Papanicolaou's stain on smears of effusion fluids. Additional techniques have included Millipore filters and Giemsa staining on air dried smears of sediments. The cytologic examination of body fluids is of distinct value in confirming or disapproving malignant metastatic tumors to the cavities. The method is more of prognostic value rather than for the early detection or prevention of further tumor growth.

MATERIALS AND METHODS: This was a prospective study which was carried out in the department of Pathology at Gandhi Medical College, Secunderabad, over a period of one year two months. In the present study, a total of 150 samples of ascitic and pleural fluids were studied which were aspirated from admitted patients of medical, surgical and gynecologic wards. In all the cases, relevant data regarding name, age, gender, registration number of the patient, type of fluid, and the provisional clinical diagnosis were noted on a predesigned proforma.

ORIGINAL ARTICLE

The fluids were equally divided and simultaneously centrifuged in ordinary Remi centrifuge and the Shandon Cytospin II. The Cytospin II is a device for depositing cells directly on to the microscope slides with an operating speed ranging from 200 to 2000 rpm. Three parameters, speed, time and acceleration rate are programmable. The selected parameters once selected are held in a volatile memory. Samples are centrifuged in special plastic chambers, each sample chamber assembly comprising of sample chamber, special filter card and glass microscope slide are held in position by using special stainless steel slide clip. Twelve of these sample chamber assemblies fit into the sealed head.

The principle is based on the fact that because the cell is denser than the suspending fluid, under an applied force the cell will have greater momentum than the fluid. This means that after passing through the sample chamber the cells will be projected towards the microscope slide with sufficient momentum. The suspended fluid is absorbed by the filter card by capillary action and the cells are deposited on the microscope slide.

Fresh samples were used which were recently collected. No preservatives were used so they were likely to contain substantial proportion of cells with well-maintained morphology. The smears for both methods were fixed for hematoxylin and eosin stain. Other stains like Pap and PAS (Periodic acid Schiff) were done only for those cases which were positive for malignancy. Delay in processing was avoided as far as possible but wherever delay was anticipated, the samples were kept in refrigerator at 4° Centigrade. No fixatives or anticoagulants were added to the specimen. Fluids which were gelatinous or viscid were not included in the study because of difficulty in centrifuging them in the Cytospin.

Fluids were centrifuged at 1000 rpm for 10 minutes in Cytospin II. In the Remi centrifuge the speed and duration used were 1500 rpm and 5 minutes respectively. After staining and mounting, the slides were examined under the microscope. The cytology smears were correlated with histopathology whenever it was possible.

RESULTS: A total of 150 randomly obtained specimens [57(38%), pleural and 93(62%), peritoneal fluids] were studied by processing the samples simultaneously in ordinary centrifuge and Cytospin II. There were 68 male and 82 female patients and the ratio was 0.8:1. (Table 1)

The fluids were classified as non-specific inflammatory, specific inflammatory like tuberculous, suspicious for malignancy and frankly malignant effusions. (Table 2). Transudates were diagnosed when ordinary centrifuge preparation showed few mesothelial cells and inflammatory cells, whereas the Cytospin preparation showed plenty of mesothelial cells. Exudates showed neutrophils, chronic inflammatory cells and mesothelial cells. Tuberculous type was diagnosed when the predominant cell population was lymphocytes with relative absence of mesothelial cells. Effusions suspicious of malignancy were those which fell short of typical malignant change. Malignant effusions were diagnosed when clusters of pleomorphic cells with hyperchromatic, enlarged nuclei showed irregular chromatin, pleomorphism and prominent nucleoli.

Among 57 pleural fluids examined, there were 30 males and 27 females, the youngest being 21 years and oldest being 60 years. (Table 3) There were 9(15.78%) cases of malignant pleural effusions of which 8(14.03%) were adenocarcinomas and one (1.75%) was a case of Non-Hodgkin's lymphoma in a 21 year old male whose lymph node biopsy later confirmed diffuse lymphoblastic lymphoma. In the adenocarcinomas, ovary was the primary site in two cases, whereas in 5 cases the

ORIGINAL ARTICLE

site could not be identified. In three cases there was ascites which also showed similar malignant features and one was diagnosed to have hepatocellular carcinoma.

For the peritoneal fluids there were 38 male and 55 female patients. Two cases were suspicious for malignant cells. (Table 4) Sixteen (17.20%) cases showed definite cytological evidence of malignancy 13 being female and 3 were males. There were 12(12.90%) cases of ovarian malignancy with peritoneal effusion. Two of these cases had associated pleural effusion with similar cytological features. Two cases also showed PAS positive material within the cells. In six cases (50%) histopathological confirmation was available. Four cases had features of papillary adenocarcinoma and one case each had granulosa cell tumor and epithelioid leiomyosarcoma.

There were three cases of gastric carcinoma with malignant hemorrhagic peritoneal effusion, two of them were males aged 60 years and one was a female aged 28 years. A single case of hepatocellular carcinoma was diagnosed in a 48 year old woman with massive ascites and the patient expired on 5th day of hospital admission.

Technique wise the following advantages were observed in the Cytospin method, which are as follows- It requires little time of the operator, the cell loss is minimal, it reduces the screening time of the slides, centrifugal force constructively flattens individual cells which enhances the display of their chromatin distribution patterns, it produces cytological preparation that can be air dried or wet fixed in any type of fixative for any type of stain, it reproducibly yields high quality results, requires no special handling after cytocentrifugation and protects against release of potentially harmful bacteriologic aerosols during processing.

DISCUSSION: Cytologic examination of body fluid is of distinct value in confirming or disapproving malignant metastatic tumors to the cavities. Since mesothelial and synovial tumors are rare, this method is useful to detect metastatic malignant cells to the body cavities.² Ratio of pleural to peritoneal fluids in the present study was 38:62, whereas in other studies it has been variously reported as 66:34 (Jhonson WD),³ and 85:15 (Lopes-Cardozo PL).⁴ This is perhaps because of the higher incidence of ovarian carcinomas in our material. In other studies the primary site of malignancy was mainly the breast and lung and hence the pleural cavity was more frequently involved.

The cells were scanty, especially in cases of transudates and it was difficult to study the morphology of cells by an ordinary centrifuge sediment preparation. Moreover the cells were spread out over a large area taking much time to screen the slide. In Cytospin II preparation the cells were concentrated in an area of 32 Sq.mm The morphology of cells was well-preserved and even one or two drops of effusion fluid was sufficient to make smears and give good information. In our experience, even the samples stored at 4° C overnight showed cells that retained their morphology to a considerable extent. Fresh samples offer several important advantages compared to samples collected in preservatives. They are easier to handle, cells are stickier and can adhere well to the glass slide as they have not been hardened by preservatives and cell recovery is greater. All these features have been noted in our study.

Two cases (8%) of malignant effusions were missed on smears prepared from the deposit obtained by ordinary centrifuge. The malignant cells were spotted by cytospin method.

The fluids act as good culture media and exfoliated cells benign or malignant can continue to live and divide in the fluids which explain why cells in mitosis are more likely to be seen in effusions

ORIGINAL ARTICLE

than in any other type of tissues. Multinucleation and mitoses were seen in 90% of our cases. One should not give undue importance to mitosis in effusion fluid and over diagnose malignancy. The diagnosis of malignancy solely rests on nuclear features in effusions. Sometimes it is difficult to differentiate reactive mesothelial cells from malignant cells. Mesothelial cells in fluids vary from one disease to another. In tuberculous effusions the mesothelial cells are scanty. This is due to severe inflammatory reaction destroying the mesothelial cells. Often the mesothelial surface is covered by fibrin which is abundant in tuberculosis. This prevents shedding of mesothelial cells into the cavity.^{5,6,7} However, this cytological evidence should be confirmed by biopsy and staining for acid fast bacillus.⁸

In our study, we had 12 cases (8%) of tuberculous effusions, 8(66.6%) from pleural and 4(33.33%) from peritoneal cavities. They showed predominantly lymphocytes. The chromatin of the lymphocytes was splintered and scattered throughout the nucleus and there were very few mesothelial cells. In 2 cases biopsy from omentum and lymph node confirmed the diagnosis. In rest of the cases there was therapeutic response to antituberculous drugs, thereby showing the effusions were of tuberculous nature. Luse et al,⁹ have noted Langhans type giant cells in 31% of congestive heart failure patients and in 32% of cirrhosis cases. In our study also we observed a case of chronic obstructive pulmonary disease with congestive heart failure whose pleural effusion showed plenty of Langhans type giant cells.

In our study, 15% of pleural and 17% of peritoneal fluid specimens were positive for malignant cells. Sears et al,¹⁰ reported 44% and 36% positivity for malignant cells in pleural and peritoneal fluids respectively. Lopes Cardozo,⁴ has reported higher incidence of malignancy in peritoneal fluids as compared to pleural fluids. The most frequent neoplasms leading to malignant pleural effusion in decreasing order of frequency were pulmonary carcinoma (21%), mammary carcinoma (16%), mesothelioma (10%), lymphoma (5%), ovarian carcinoma (4%), gastric (3%) and pancreatic carcinoma (2%).⁴ In other studies it was pulmonary¹¹ and breast carcinoma.^{10,12} In our study, the primary site of malignancy in order of frequency was ovary (20%), hepatocellular (10%), lymphoma (10%) and unidentified primary (60%).

It is interesting to note that out of 9 malignant pleural effusions, 8 were females and none of them had any breast malignancy. Our study does not concur with the high incidence of breast carcinoma leading to malignant pleural effusion in other studies quoted earlier, inspite of fairly high incidence of breast carcinoma reported in our hospital. This is explained by the fact that once carcinoma breast is reported either by biopsy or cytology they are referred to Cancer Centre's and are lost to follow up.

As quoted by Light et al,⁵ Osler reported that 95% of blood tinged pleural effusions were malignant. In our study 80% of malignant pleural effusions and 50% of peritoneal effusions were hemorrhagic.

The frequency of diagnosis of Non-Hodgkin's lymphoma in pleural effusions shows a wide variation ranging from 14% to 86%.¹³ In our study, we encountered only one case (0.66%) of Non-Hodgkin's lymphoma.

In the present study, Ovarian carcinomas contributed 68%, gastric 18%, lymphoma 6% and hepatoma 6% to malignant peritoneal effusions. This is somewhat comparable to the figures of ovarian carcinoma 32%, gastric 23%, colonic 9%, mammary 9%, pancreatic 8% and hepatocellular 5% as reported by Lopes-Cardozo et al.⁴ Prognosis of ovarian cancer patients is worse with positive peritoneal cytology than those without.¹⁴

ORIGINAL ARTICLE

In our study the diagnostic accuracy in identifying the primary site from malignant effusion was 50% for ovarian primary which is similar to findings of Foot et al¹⁵ of 58%.

CONCLUSION: The overall percentage positivity for malignancy was 16% (25 out of 150 cases). The commonest primary in females was ovary and in males it was gastrointestinal tract.

The comparative study between the Cytospin II and ordinary centrifuge revealed that the Cytospin II preparation gives a better yield of cells specially when they are scanty, morphology of cells is very well preserved and much time is saved in screening the slide. The Cytospin II is better in picking up malignant cells than the ordinary centrifuge.

REFERENCES:

1. Mandelbaum FS. The diagnosis of malignant tumors by paraffin sections of centrifuged exudates. *J Lab Clin Med* 1917, 2: 580.
2. Foot NC. Identification of neoplastic cells in serous effusions. Critical analysis of smears from 2029 persons. *Am J Pathol* 1956, 32: 961-977.
3. Johnson WD. The cytologic diagnosis of cancer in serous effusion. *Acta Cytol* 1966, 10: 161-172.
4. Lopes-Cardozo PL. A critical evaluation of 3000 cytologic analysis of pleural fluid, ascitic fluid and peritoneal fluids. *Acta Cytol* 1966, 10: 455-460.
5. Light RW, Erozan YS, Ball WC. Cells in pleural fluid and their value in differential diagnosis. *Arch Int Med* 1973, 132(6): 859-860.
6. Yam LT. Diagnostic significance of lymphocytes in pleural effusions. *Ann Int Med* 1967, 66: 972-982.
7. Spriggs AI, Boddington MM. Absence of mesothelial cells from tuberculous pleural effusions. *Thorax*, 1960, 15: 169-171.
8. Spieler P. The cytologic diagnosis of Tuberculosis in pleural effusions. *Acta Cytol* 1979, 23: 374-379.
9. Luse SA, Regan JW. A histologic study of effusions. *Cancer* 1954, 7: 1155-1181.
10. Sears D, Hajdu ST. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. *Acta Cytol* 1987, 31: 85-97.
11. Johnston WW. The malignant pleural effusion. A review of cytopathologic diagnosis of 584 specimens from 472 consecutive patients. *Cancer* 1985, 56: 905-909.
12. Raju RN, Kardinal CG. Pleural effusions in breast carcinoma – analysis of 122 cases. *Cancer* 1981, 48: 2524-2527.
13. Das DK, Billingham ME, Melamed R. Pleural effusions in Non-Hodgkin's lymphoma. Cytomorphologic, cytochemical and immunologic study. *Acta Cytol* 1987, 31: 119-124.
14. Yosimura S, Scully RE, Bell DA, Taft PD. Correlation of ascitic fluid cytology with histologic findings before and after treatment of ovarian cancer. *Am J Obs and Gynecol* 1984, 148: 716-721.
15. Foot NC. Identification of types and primary sites of metastatic tumors from exfoliated cells in serous fluids. *Am J Pathol* 1954, 30: 661-677.

ORIGINAL ARTICLE

Age (yrs)	Pleural Fluids			Peritoneal Fluids		
	Male	Female	Total	Male	Female	Total
11-20	-	-	-	-	1	1
21-30	8	5	13	8	10	18
31-40	6	6	12	6	15	21
41-50	7	4	11	12	18	30
51-60	9	12	21	12	11	23
Total	30	27	57	38	55	93

Table 1: Age and sex distribution of 150 cases

Pleural Fluids		Peritoneal Fluids	
Tansudate	8	Tansudate	10
Non-specific inflammation	32	Non-specific inflammation	61
Tuberculous	8	Tuberculous	4
		Suspicious for malignancy	2
Malignant	9	Malignant	16
Total	57	Total	93

Table 2: Classification of lesions in fluids

Age (yrs)	Male	Female	Total
11-20	-	-	-
21-30	1	-	1
31-40	-	1	1
41-50	-	4	4
51-60	-	3	3
Total	1	8	9

Table 3: Age and sex distribution of malignant pleural effusions

Age (yrs)	Male	Female	Total
11-20	-	-	-
21-30	-	2	2
31-40	-	3	3
41-50	1	1	2
51-60	2	7	9
Total	3	13	16

Table 4: Age and sex distribution of malignant peritoneal effusions

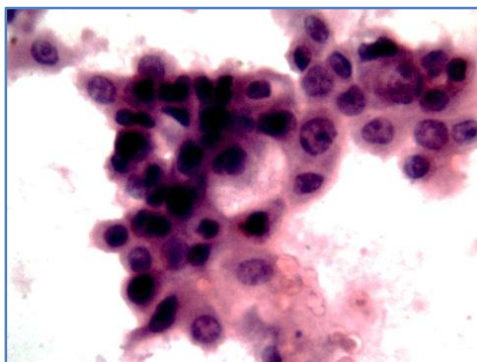


Fig. 1: Sheets of round to oval cells with pleomorphic nuclei. Pleural fluid from a case of carcinoma breast (Hematoxylin and eosin 400 X)

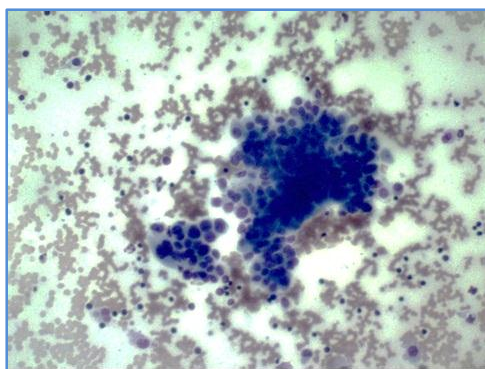


Fig. 2: Sheets and clusters of round to oval cells with pleomorphic nuclei. Peritoneal fluid from a case of adenocarcinoma ovary (Giemsa stain 40 X)

AUTHORS:

1. Ashok Kumar Deshpande
2. Syamala Bhaskaran

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Pathology, Kamineni Academy of Medical Sciences & Research Centre, L. B. Nagar, Hyderabad.
2. Retired Professor & HOD, Department of Pathology, Gandhi Medical College, Hyderabad.

FINANCIAL OR OTHER

COMPETING INTERESTS: None

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Ashok Kumar Deshpande,
Associate Professor,
Department of Pathology,
Kamineni Academy of Medical Sciences &
Research Centre, L. B. Nagar,
Hyderabad-500068.
E-mail: akdeshpande57@gmail.com

Date of Submission: 24/04/2015.
Date of Peer Review: 25/04/2015.
Date of Acceptance: 08/05/2015.
Date of Publishing: 15/05/2015.