HOW ESSENTIAL IS CELL BLOCK PREPARATION IN EACH AND EVERY SPECIMEN OF BODY FLUID SENT FOR CYTOLOGY IN ADDITION TO CONVENTIONAL SMEAR- AN OBSERVATIONAL AND ANALYTICAL STUDY IN A TERTIARY CARE CENTRE

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ABSTRACT

BACKGROUND

Cytological evaluation of effusion fluid has become integral part of management especially in suspected malignant cases. Conventional Cytosmear (CS) is relatively simple, but sometimes become challenging to differentiate between reactive mesothelial cells and malignant cells. Hence, Cell Block (CB) preparation is increasingly gaining popularity for management, though it is time consuming, costly and little more cumbersome.

MATERIALS AND METHODS

All the fluid samples received in the departmental laboratory were divided into two parts for Leishman-Giemsa and Papanicolaou (PAP) stained conventional cytosmear and Haematoxylin-Eosin stained cell block preparation. Diagnoses obtained by CS and CB method were statistically analysed.

RESULTS

Out of total 115 fluid samples, 84 were pleural fluid, 24 ascitic fluid and 7 pericardial fluids. 9 cases in pleural fluid and 6 cases in ascitic fluid were false-negative by CS, which were proved to be malignant by CB method. So, additional 13.1% yield were obtained by CB method to diagnose malignant cases.

CONCLUSION

CB is superior to CS for diagnosis of malignant effusions and hence it should be used in adjunct to CS in difficult or suspected malignant cases for better yield.

KEYWORDS

Malignant, Effusion Cytology, Cell Block.

HOW TO CITE THIS ARTICLE: Barui G, Talukdar M, Sarkar M. How essential is cell block preparation in each and every specimen of body fluid sent for cytology in addition to conventional smear- An observational and analytical study in a tertiary care centre. J. Evid. Based Med. Healthc. 2017; 4(94), 5754-5758. DOI: 10.18410/jebmh/2017/1158

BACKGROUND

Accumulation of fluid occurs in different body cavities in various non-neoplastic as well as neoplastic conditions. The major purpose of cytological examination of effusion fluid is to determine whether malignant cells are present or not. This is an extremely important task, since in most cases, the presence of malignant cells in effusion indicates an advanced or terminal stage of malignancy and it is associated with poor survival. Cytological study of body fluid is a complete diagnostic modality to find out the aetiology specially to rule out malignancy.[1] Usually, four types of cells can be found in a body fluid specimen- inflammatory cells, histiocytes, mesothelial cells and malignant cells.

Financial or Other, Competing Interest: None.
Submission 16-11-2017, Peer Review 28-11-2017,
Acceptance 11-12-2017, Published 12-12-2017.
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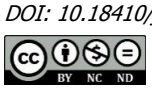
DOI: 10.18410/jebmh/2017/1158



The accurate identification and differentiation of cells as either malignant or reactive mesothelial cells is a major diagnostic challenge in conventional Cytological Smears (CS). Differentiation between benign and malignant cellular changes may require meticulous screening, careful scrutiny of cellular features and realising the range of reactive changes due to various reasons. There are several factors that might cause lower diagnostic yield in CS method like cellular overlapping, delaying artifact, suboptimal processing, preparatory cytotechnique and leaving behind useful material.

The Cell Block (CB) technique is one of the oldest and complementary methods for the evaluation of body cavity fluids. The main advantages of the CB technique are preservation of tissue architecture and opportunity of obtaining multiple sections for other ancillary tests like special stains and immunohistochemistry, if necessary.[2] However, it is time consuming as the histopathological processing is required as well as costly in comparison to conventional cytosmear.

In our study, we have tried to depict a comparative picture between the conventional cytosmear and cell block

preparation of all types of fluid specimen sent for cytology to find out the utility of preparing a cell block in each and every specimen of fluid cytology in addition to conventional smear.

Aims and Objectives

To depict a comparative picture between the conventional cytosmear and cell block preparation of all types of fluid specimen sent for cytology to find out the utility of preparing a cell block in each and every specimen of fluid cytology in addition to conventional smear.

MATERIALS AND METHODS

This cross-sectional observational study was done in the Department of Pathology, R.G. Kar Medical College, Kolkata, in association with Central Laboratory Department of same institution. Total duration of study is one year starting from April 2016 to March 2017. Whenever a fluid specimen is received, it is divided into two parts and transferred into two test tubes. One sample is centrifuged in 3000 rpm for 5 minutes and supernatant is discarded, the centrifuged deposit poured into slides and air-dried and alcohol-fixed conventional cytosmears are prepared for Leishman-Giemsa and Papanicolaou (PAP) stain, respectively. Another sample is kept ready for Cell Block (CB) preparation.

Method of Cell Block Preparation- 20-30 mL of material is centrifuged for 2 mins. at 1500 rpm to obtain a cell button. Supernatant is decanted and 5 mL alcohol formalin solution (9 part 95% alcohol and 1 part 10% formalin) is added to it. After one hour, this is centrifuged for 10-20 minutes at 1500 rpm. Cell block cassette is prepared with the patient's name and case number. Now, the supernatant is decanted and cell button from the centrifuge tube is removed. A second centrifugation with 95% alcohol may be needed if the cell button is not hard. When the cell button is free, it is lifted from the bottom of the tube and placed it on tissue paper. Tissue paper with cell button is folded and placed in cassette. Cell block is fixed in formalin and processed at histology laboratory along with other routine histological specimens.

RESULTS

Total 84 cases of pleural fluid, 24 ascitic fluid and 07 cases of pericardial fluid were analysed. The mean (± SD) age of pleural, peritoneal and pericardial effusion cases were 52.7 (±16.9) (range16 to 82 years), 53.1 (±3.5) (range 47 to 60 years] and 46.2 (±14.0) (range 32 to 61 years), respectively. Out of 84 patients having pleural effusion, 53 (63.1%) were male and 31 (36.9%) were female. In case of peritoneal effusion, 7 (29.2%) cases were male and rest 17 (70.8%) cases were female while in case of pericardial effusion, 5 (71.4%) cases were male and other 2 (28.6%) were female patients. The findings regarding diagnosis by conventional cytosmear and cell block preparation in pleural, peritoneal and pericardial effusion cases are elicited in table 1, 2 and 3, respectively.

<table>
<tr><th rowspan="3">Total Number of Pleural Fluid</th><th colspan="3">Conventional Cytosmear (CS) Findings</th><th colspan="3">Cell Block (CB) Findings</th><th rowspan="3">Number of Mismatch</th></tr>
<tr><th colspan="2">Non-Neoplastic (Inflammatory and Mesothelial Cell Hyperplasia)</th><th>Neoplastic (Suspicious/ Positive Smear of Malignancy)</th><th colspan="2">Non-Neoplastic (Inflammatory and Mesothelial Cell Hyperplasia)</th><th>Neoplastic (Suspicious/ Positive for Malignancy)</th></tr>
<tr><td rowspan="3">84</td><td colspan="2">60</td><td rowspan="3">24</td><td colspan="2">51</td><td rowspan="3">33</td><td rowspan="3">9 cases where CS showed mesothelial cell hyperplasia and CB showed positive for malignancy</td></tr>
<tr><td>Inflammatory</td><td>Mesothelial cell hyperplasia</td><td>Inflammatory</td><td>Mesothelial cell hyperplasia</td></tr>
<tr><td>40</td><td>20</td><td>40</td><td>11</td></tr>
</table>

Table 1. Analysis of Pleural Fluid Cytology to Detect Discrepancies between Cytosmear (CS) and Cell Block (CB)

<table>
<tr><th rowspan="3">Total Number of Ascitic Fluid</th><th colspan="3">Conventional Cytosmear(CS) Findings</th><th colspan="3">Cell Block (CB) Findings</th><th rowspan="3">Number of Mismatch</th></tr>
<tr><th colspan="2">Non-Neoplastic (Inflammatory and Mesothelial Cell Hyperplasia)</th><th>Neoplastic (Suspicious/ Positive Smear of Malignancy)</th><th colspan="2">Non-Neoplastic (Inflammatory and Mesothelial Cell Hyperplasia)</th><th>Neoplastic (Suspicious/ Positive for Malignancy)</th></tr>
<tr><td rowspan="3">24</td><td colspan="2">19</td><td rowspan="3">5</td><td colspan="2">13</td><td rowspan="3">11</td><td rowspan="3">6 cases where CS showed mesothelial cell hyperplasia and CB showed positive for malignancy</td></tr>
<tr><td>Inflammatory</td><td>Mesothelial cell hyperplasia</td><td>Inflammatory</td><td>Mesothelial cell hyperplasia</td></tr>
<tr><td>11</td><td>8</td><td>11</td><td>2</td></tr>
</table>

Table 2. Analysis of Peritoneal/Ascitic Fluid Cytology to Detect Discrepancies between Cytosmear (CS) and Cell Block (CB)

Total Number of Pericardial Fluid	Conventional Cytosmear(CS) Findings			Cell Block (CB) Findings			Number of Mismatch
	Non-Neoplastic (Inflammatory and Mesothelial Cell Hyperplasia)		Neoplastic (Suspicious/ Positive Smear of Malignancy)	Non-Neoplastic (Inflammatory and Mesothelial Cell Hyperplasia)		Neoplastic (Suspicious/ Positive for Malignancy)	
7	5		2	5		2	Nil
	Inflammatory	Mesothelial cell hyperplasia		Inflammatory	Mesothelial cell hyperplasia		
	3	2		3	2		

Table 3. Analysis of Pericardial Fluid Cytology to Detect Discrepancies between Cytosmear (CS) and Cell Block (CB)

Diagnostic Category	CS Method	CB Method
Non-Neoplastic	84 (73.1%)	69 (60%)
Suspicious/positive for malignancy	31 (26.9%)	46 (40%)
Total	**115 (100%)**	**115 (100%)**

Table 4. Comparison of Cytosmear (CS) and Cell Block (CB) Method in Total 115 Fluid Samples

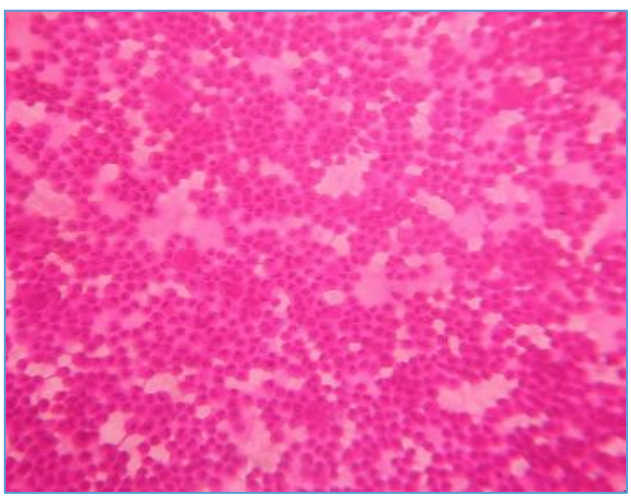


Figure 1. Cell- Block Preparation Showing Plenty of Inflammatory Cells (Haematoxylin-Eosin Stain, 400X)

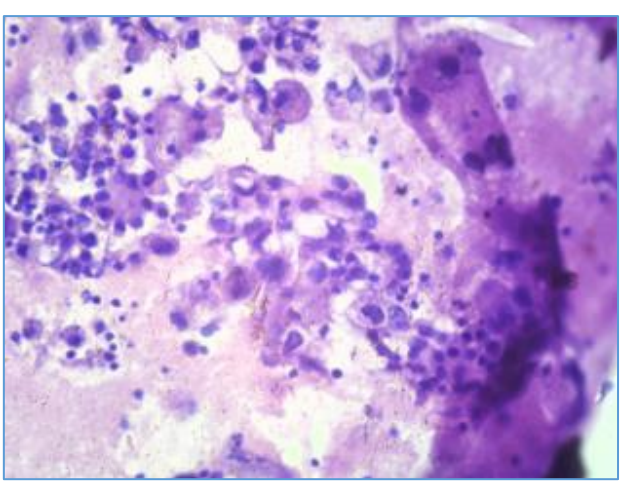


Figure 2. Cell Block Preparation Showing Clusters of Malignant Epithelial Cells in a Pleural Fluid Sample (Haematoxylin-Eosin Stain, 400X)

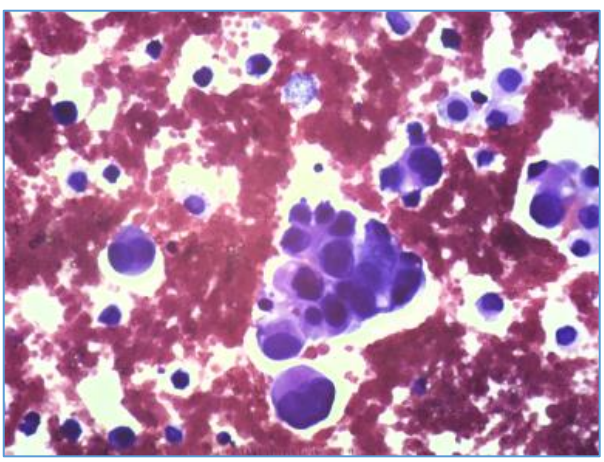


Figure 3. Conventional Cytosmear Showing Clusters Suspicious of Malignant Cells in a Pericardial Fluid Sample (400X, PAP Stain)

DISCUSSION

The cytological examination of effusion fluid has increasingly gained acceptance regarding diagnostic approach among clinicians to such an extent that a positive diagnosis is often considered the definitive test and obviates the need of explorative surgery for further treatment planning. It helps not only for the diagnosis of malignant lesions, but also important for staging and prognosis.[3] The development of malignant pleural and peritoneal effusion is a common complication of advanced stage of different cancers like pulmonary, gastric ovarian and colon carcinomas.[4] Not only in adult patients, examination of fluids from the serous cavities of the body has become an essential component of management in paediatric cases too. Malignant neoplasms, especially lymphoid neoplasms represent a major cause of cancer-related death in children, and in these cases, cytological examination of body fluid has become integral part of the management.[5]

One of the most common problems in CS cytology is to distinguish reactive mesothelial cells from metastatic deposits, especially adenocarcinoma. The difficulty is either due to marked atypical mesothelial cells caused by the

different insults to the serous membranes like microbiological, chemical, physical, immunological or metabolic or due to the subtle cytomorphological features of some malignant neoplasms, particularly well-differentiated adenocarcinomas. The problem may become further complicated by artifacts from poor fixation, preparation or staining techniques.[6] Although, the preparation of CS is a much simpler and cost-effective procedure than that of CB, it has a few limitations like lack of tissue architecture, leaving behind useful material, etc. In some situation, appreciations of tissue architecture help to diagnose the difficult cases.[7] Moreover, conventional cytological examination of effusion fluids has a sensitivity of only 40-70% to detect the presence of malignant disease due to overcrowding of cells, cell loss and different laboratory processing methods. Presence of reactive mesothelial cells, abundant inflammatory cells and paucity of representative neoplastic cells contribute to considerable difficulties in making conclusive diagnosis on conventional smears.[8]

Since the invention of the CB technique by Bahrenburg in 1896, it has been used extensively for examination of body fluids.[9] In 1928, Zemansky expressed that the CB method was superior to the CS technique.[10] Cancer cells in the pleural or peritoneal fluid almost always point towards metastatic cancer as tumours arising from mesothelial cells, lining these spaces are rare. When present, the tumour cells are usually numerous and frequently in clusters maybe found. The findings of glandular forms and demonstration of mucin in the tumour cells are more reliable evidence on CB to diagnose adenocarcinoma.[11] Diagnostic difficulties arise whenever there is only marginal morphological distinction; for example, between reactive atypia of mesothelial cells and poorly-differentiated malignant cells.[10,12]

In our study, the total number of body fluid specimen received 115, of which, 84 (73%) was pleural fluid, 24 (20.9%) was ascitic fluid and 7 (6.1%) cases of pericardial fluid.

Since, the pleural fluid comprises the major group, if we analyse the Table 1, we will find that out of 84 specimens of pleural fluid, 60 are non-neoplastic including both inflammatory smear (40) (Figure 1) and reactive mesothelial hyperplasia (20) by CS method. There is no discrepancies of cytological findings of inflammatory smears both in conventional cytosmear and cell block preparation. Whereas, if we consider the reactive mesothelial cells, which comprise 20 cases by CS method, there is definite mismatch of cytological finding between the conventional smear and cell block preparation. 9 cases which were reported as reactive mesothelial cells in conventional smear, later on showed the feature of metastatic adenocarcinoma (Figure 2) in cell block study. This study corroborates the study of Shivakumarswamy U et al.[10] They showed the discrepancies of 7 cases between conventional smear and cell block study where the 7 cases diagnosed as benign in CS, later on diagnosed as malignant in CB study.

In all other cases of neoplastic group, 24 cases which were reported as suspicious and positive smear for malignant cells, no discrepancies were noted between conventional smear and cell block study.

In case of ascitic fluid, the total 24 number of specimens were received. If we analyse the Table 2, we will find 19 cases were non-neoplastic and 5 cases were neoplastic by cytosmear findings. Of non-neoplastic group, only 6 mismatches were detected in the category of reactive mesothelial cell hyperplasia, which was later on diagnosed as positive smear for malignant cell in cell block preparation. The findings are supported by previous study of Shivakumarswamy U et al where they showed additional 6 cases detected as malignant by CB method.[9] On the contrary, no mismatch was found in the neoplastic group where the conventional smear reported as positive or suspicious for malignant cell. All the cases were proved to be positive for malignancy by cell block preparation also.

In case of pericardial fluids, the total number of specimen received was 7. Out of 7 cases, 5 cases were non-neoplastic and 2 cases neoplastic (Figure 3). If we analyse the Table 3, we will find that there is no mismatch of cytological finding between conventional cytosmear and cell block preparation in both non-neoplastic and neoplastic group.

Analysing Table 4, we find that, out of total 115 fluid samples, 46 cases (40%) were diagnosed as malignant by CB method and 31 (26.9%) were diagnosed as suspicious/positive for malignancy by CS method. So, by CB method, additional 15 cases were detected as positive for malignancy that is 13.1% more diagnostic yield for malignancy. Similar findings were observed by Viral MB et al,[12] where they analysed 150 fluid samples and showed 10% additional yield for malignancy by CB method. Shivakumarswamy U et al also showed additional yield of 13.63% for malignancy by CB method in their analysis on 44 ascitic fluid samples.[9] Shivakumarswamy U et al did another study on pleural fluid where they revealed additional yield for malignancy was 15% more by CB method.[10] Another study was done by Niveditha SR et al and the study was conducted on comparative study between conventional smear and cell block preparation of the fluid specimen of cystic lesions. They showed that CB gave an improved diagnosis in 20% cases of malignancy and concluded CB complemented CS, especially in malignant cases.[13] Nair GG et al concluded that both CB and CS had high specificity, but sensitivity of CB is greater than CS to detect malignancy.[14]

CONCLUSION

From this study, we may definitely draw a conclusion that as far as fluid cytology is concerned, cell block preparation has superior position than that of conventional smear specially to differentiate reactive mesothelial cells from metastatic adenocarcinoma as well as to perform immune histochemical study where it will be necessary to substantiate the diagnosis. Hence, CB technique can be used as complementary investigation in addition to conventional cytosmear examination especially in difficult cases to differentiate reactive mesothelial cells from metastatic

adenocarcinoma as CB method has better diagnostic yield for detecting malignancies.

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