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Diagnostic utility of cytospin in comparison to cell block in peritoneal and pleural fluid cytology

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ABSTRACT

Background: Fluid cytology plays an important role in delineating benign from malignant effusions, tumor staging, and also in diagnosing recurrences. Various methods are used in cytology for the preparation of smears. As the accurate diagnosis of the fluids aids in clinical decisions, the method of preparation of cytology smears, it is very important. Cytospin preparation of smears is one of the methods which provide higher cellular yield with better preservation of cellular morphology and is less time consuming. On the other hand, cell block method gives superior architectural details and provides options for immunocytochemistry. **Aims and Objectives:** The aim of this study was to assess the diagnostic utility of cytospin in comparison to cell block method in peritoneal and pleural fluid cytology. The study is done to determine the sensitivity, specificity, predictive values, and diagnostic accuracy of cytospin preparations with cell block method which is considered as the gold standard. **Materials and Methods:** This was a diagnostic test evaluation study done at the Department of Pathology, Government Medical College, Kottayam. The sample size was 240 which included all pleural and peritoneal fluids received in our cytology laboratory during the study period. Cytospin prepared smears of peritoneal and pleural fluids were compared with the tissue sections prepared by cell block method, to evaluate the diagnostic utility of cytospin. Tissue sections prepared from the cell blocks of effusions were considered as the gold standard for comparison. **Results:** A diagnostic test evaluation of cytospin preparation was done with cell block preparations. The sensitivity of cytopsin preparations in pleural and peritoneal fluid cytology is 94%. The specificity of cytopsin preparations in pleural and peritoneal fluid cytology is 100%. The positive predictive value of cytopsin preparations in pleural and peritoneal fluid cytology is 100% and the negative predictive value of cytopsin preparations in pleural and peritoneal fluid cytology is 96.8%. Hence, accuracy of the test is 97.9%. **Conclusion:** There is only minimal statistical difference between the results obtained by the cytospin and cell block methods. Cytospin method is less time consuming along with the advantage of higher cellular yield. Hence, the incorporation of cytospin along with the cell block technique is beneficial for augmenting the results of effusion cytology.

Key words: Diagnostic utility; Cytospin; Cell block; Predictive value; Accuracy

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INTRODUCTION

Serous effusions are characterized by accumulation of fluid in excess of the normal amount, which may be derived from the plasma or from the mesothelial cells lining the peritoneal, pleural, or pericardial cavities. Fluid collection other than blood in these cavities results in effusion. Accumulation of fluid in the peritoneal cavity is called ascites. Effusions are classified based on specific gravity and protein content into two types – transudates and exudates.[1]

Effusion cytology is the science of interpretation of cells which are exfoliated from the epithelial surfaces or removed from various tissues. Cytological study gives

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the first indication of malignancy in about one-third of malignant effusions. Fluid cytology is a non-invasive, simple technique that helps in faster reporting and is relatively inexpensive. Accurate diagnosis of cells of serous fluids is a major challenge in distinguishing benign from malignant effusions and may require meticulous screening.[2] It is very difficult to achieve due to bland morphological details of cells in many cases, cell loss, overcrowding, or overlapping of cells and due to different processing methods used by the laboratories.[3] The cytological examination of serous effusions is significant as it provides a definitive diagnosis that helps in staging, prognosis, and management of the patients with various malignancies. It also provides information about various inflammatory and non-inflammatory lesions of serous lining of the body cavities. The information provided by body fluid analysis helps the clinician in formulating therapy and prognosis.[4] There are a wide range of cytological techniques available to analyze body fluids, from simple direct smears, cytospin smears to cell block methods.[2,5]

The cytospin smear method is designed to concentrate cells, especially in serous effusions with poor cellularity, in which the cells are allowed to be spun at various speeds and times for the formation of a monolayer of cells in a limited area for the best assessment of the Cells.[6] The cell block (CB) technique is one of the oldest method for the evaluation of body cavity fluids, in which small tissue fragments in a fluid specimen are fixed and then processed to form a tissue embedded paraffin block. The advantages of cell block technique include preservation of tissue architecture and obtaining multiple sections for special stains and immunohistochemistry.[4,6] A good cell block aids in molecular diagnostic studies such as fluorescence *in situ* hybridization, polymerase chain reaction and cDNA microarray analysis.[7,8]

Aims and objectives

The present study is aimed to analyze the diagnostic utility of cytospin in comparison to cell block in peritoneal and pleural fluid cytology.

MATERIALS AND METHODS

The present study was a diagnostic test evaluation study done at the Department of Pathology, Government Medical College, Kottayam. The study was preapproved by the Institutional Ethics Committee for the final permission. The sample size was 240 which included all pleural and peritoneal fluids received in our cytology laboratory during the study period of 18 months. Fresh samples of peritoneal and pleural fluid are analyzed using both cytospin and cell block preparation. Relevant clinical details including age, sex, presenting symptoms, and clinical diagnosis are obtained. The Shandon Thermo fisher cytocentrifuge is used to prepare cytospin smears. Cytospin smears are prepared by placing 0.5 mL fluid in the cytospin funnel with filter paper being placed between the funnel and the slide, followed by centrifugation at 750 rpm for 5 min resulting in formation of a monolayered sheet of cells within a small circumference. Two such smears are prepared. One smear is air dried and stained with Giemsa stain, while the other smear is fixed in 95% ethanol and is stained by Papanicolaou (PAP) method. Cell block preparation is done by centrifugation of 5 mL of fluid for 5 min at 3000 rpm followed by fixation in alkali-activated foam (AAF) (95% ethanol+glacial acetic acid+formalin) fixative for 1 h. Then after removal of supernatant fluid, the sediment is again centrifuged. The obtained sediment is again mixed with AAF, filtered, and processed as routine histopathology to obtain cell block sections. The tissue sections are stained with Hematoxylin and Eosin (H and E). Both cytology slides and histopathology slides are studied systematically and classified.

Statistical analysis

The present study was conducted on pleural and peritoneal fluids of 240 cases, wherein the samples were studied using cytospin prepared smears and correlated with cell block method. The diagnostic utility of cytospin was compared with cell block method, being the gold standard. Data were entered in Microsoft Excel, and further, statistical analysis was done using SPSS software version 26. Sensitivity, specificity, positive predictive value, and negative predictive value of cytology diagnosis by the cytospin preparation were compared with cell block method.

RESULTS

Among the 240 cases studied, 50.4% (n=121) of patients were in the age group of 61–80 years followed by 41.3% cases who were in the age group 41–60 years. About 62.1% (n=149) cases were female. In this study, 111 (46.3%) samples were pleural fluids and 129 (53.7%) were peritoneal fluids. Two hundred and twenty-five samples were found to be adequate and 15 samples were inadequate. Inadequate samples showed blood and paucicellularity.

On studying the cytospin prepared smears under microscope, majority of cases (n=125) showed non-neoplastic findings, while 21 cases had microscopic features suspicious of malignancy. Seventy-nine cases had findings favoring malignancy and 15 cases were inadequate. Among the 125 non neoplastic lesions in cytospin smears, 72 cases (57.6%) showed reactive mesothelial

cells with inflammation. Other findings observed were acute inflammation, chronic inflammation, and cases showing reactive mesothelial cells only. Majority of neoplastic effusions diagnosed in cytospin smears were adenocarcinomas (75%, n=75) followed by two cases of round cell tumor metastasis and two cases of poorly differentiated carcinomas. Among the 75 adenocarcinoma cases, 70 cases showed malignant cells arranged in acinar pattern, clusters, and singly scattered, whereas papillary pattern was the characteristic feature in four cases. One case showed signet ring cells.

Microscopic examination in cell block sections showed 79 cases favoring a diagnosis of malignant effusion, while 21 cases showed features suspicious of malignancy. Majority of cases (n=125) had cytological features consistent with non-neoplastic effusion, while a specific diagnosis could not be offered for 15 cases due to lack of cells. Among the non-neoplastic lesions, majority (71 cases) showed reactive mesothelial cells with inflammation. Other cases studied were chronic inflammation (19 cases), reactive mesothelial cells only (14 cases), acute inflammation (10 cases), and acute on chronic inflammation (ten cases). Out of the 101 neoplastic effusions studied, 79 cases were showing features of adenocarcinoma followed by three cases with features of poorly differentiated carcinoma and two cases of round cell tumor (Table 1). Seventeen cases were reported as atypical cells suspicious for malignancy. Various histological patterns observed in the cases with malignant effusion were acinar/singly scattered in 73 cases, papillary pattern in four cases, signet ring cells, and round cells in two cases each and poorly differentiated carcinoma in three cases.

On comparing the cytospin preparation smears with cell block sections, concordance was observed in 234 cases out of the 240 cases (97.5%). Six cases showed discordance (Table 2 and Figure 1). Four cases reported as suspicious of malignancy in the cytospin preparation turned out to be adenocarcinoma on cell block study. One case diagnosed in cytospin preparation as suspicious of malignancy was reported as poorly differentiated carcinoma in cell block preparation. One case of reactive mesothelial cells with atypia reported in cytospin preparation was diagnosed to be suspicious for malignancy in the cell block.

Diagnostic utility of cytospin in comparison to cell block was analyzed in the 225 samples which included sensitivity, specificity, positive predictive value, and negative predictive value of the test (N=225 Since 15 cases were inadequate for analysis). Sensitivity and specificity were calculated as 94% and 100%, respectively. Positive predictive value and negative predictive value were calculated to be 100% and 96.8%, respectively, with an accuracy of 97.9% (Table 3).

Table 1: Comparison of morphological types of neoplasms in cytospin and cell block

Morphological types of neoplasms	Cytospin (n=79)	Cell block (n=84)
Adenocarcinoma	75 (75%)	79 (78.2%)
Round cell tumor	2 (2%)	2 (1.9%)
Poorly differentiated carcinoma	2 (2%)	3 (2.9%)

DISCUSSION

This study was aimed at evaluating the diagnostic utility of cytospin in pleural and peritoneal samples, considering cell block as the gold standard. The study was conducted on 240 patients whose fluid specimens were submitted to the Department of Pathology, Government Medical College, Kottayam.

Cytospin preparations of fluid were used that provided sufficient cell yield and showed monolayer arrangement of cells in microscopy. Cytological evaluation of fluids and histopathological evaluation of H and E stained slides were performed. The accuracy of diagnostic utility of cytospin was compared with cell block which is the gold standard.

Out of 240 patients, 121 (50.4%) patients were in the age group of 61–80 years. Minimum age was 10 years and maximum age was 86 years. Hence, most of the patients are elderly. Most of the malignant cases are in the 6th decade.[5]

In the present study, 149 patients (62.10%) were female and 91 patients (37.9%) were male, male: female ratio being 1:1.6. According to the study by Mulkalwar et al., females are affected more than males with M: F ratio of 1:1.5.[2] In the study by Joshi et al., males are affected more

Table 2: Cytospin – Cell block discordant cases with probable reason for discordance

Cytospin diagnosis	Cell block diagnosis	Probable reason for discordance
Suspicious of malignancy (one case)	Poorly differentiated carcinoma (one case)	Due to obscured cell morphology by blood cells in cytospin
Reactive mesothelial cells with atypia (one case)	Suspicious of malignancy (one case)	Architectural details better visualized in cell block
Suspicious of malignancy (four cases)	Adenocarcinoma (four cases)	Architectural details are better appreciated in cell block. Ancillary techniques such as IHC aided in diagnosis

than females.[6] In the present study also, malignant cases are more in females.

In the study, comparison of results between cytospin and cell block was made in 240 cases (Table 4). Cytospin preparation identified 79 cases as malignant, whereas cell block could diagnose additional five cases (2.1%) of malignant etiology in fluids. Our study is in concordance with the study of Sidhu et al., where cell block diagnosed additional 7 (6.4%) malignant cases in effusions.[4] There is no much statistical differences in non-neoplastic lesions in cytospin and cell block. One case of non-neoplastic lesion in cytospin is diagnosed as suspicious of malignancy in cell block. Out of 240 cases, six cases show discordancy in diagnosis between cytospin and cell block.

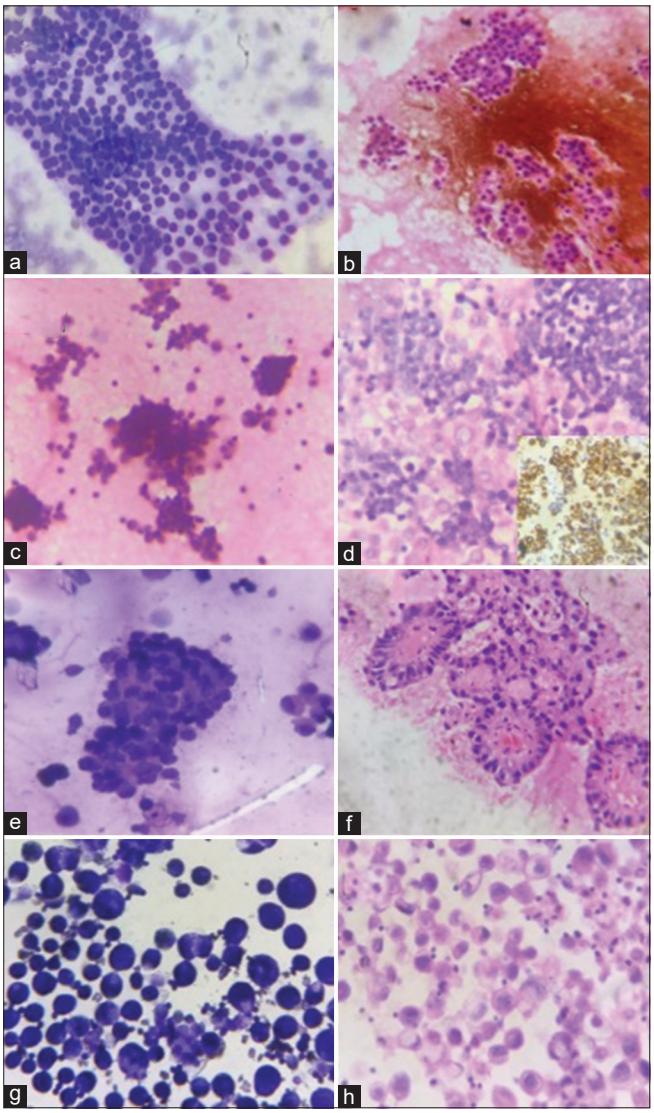

Figure 1: (a) Reactive mesothelial cells in sheets Giemsa stain ×40. (b) Reactive mesothelial cells cell block ×40. (c) Malignant round cell tumor Papanicolaou stain ×40. (d) Malignant round cell tumor cell block (H and E stain ×40) inset- IHC showing cytoplasmic Desmin positivity. (e) Suspicious of malignancy Giemsa stain ×40. (f) Adenocarcinoma in cell block ×40. (g) Suspicious of malignancy Giemsa stain ×40. (h) Poorly differentiated carcinoma cell block ×40

In the present study, non-neoplastic cases 124 (52.1%) predominates and shows concordance with studies of Mulkalwar et al., and Sidhu et al.[2,4] Out of 240 cases, 84 cases (35%) are malignant cases in the present study, but in the study by Mulkalwar et al., out of 170 cases 39 (22.9%) are malignant.[2] In the study of Sidhu et al., out of 240 cases, 34 (14.1%) cases are malignant.[4] The present study detected more malignant cases than the study by Mulkalwar et al., and Sidhu et al.[2,4]

Adenocarcinoma is the most common malignancy in fluids in the present study which is in concordance with the studies of Mulkalwar et al., and Irmeen et al.[2,9] Manifestation of glandular formations and acinar groupings is better appreciated in cell block method than cytospin preparations. Two cases of malignant round cell tumors were identified in cytospin which was diagnosed as Rhabdomyosarcoma and Non-Hodgkin's lymphoma by cell block with the aid of immunohistochemistry (Figure 1).

In the present study, specificity and positive predictive value is 100% which is in concordance with the study of Nounechutuo et al. (Table 5).[5] The sensitivity of the present study is 94% and NPV is 96.8%.

Cytological diagnosis of effusions has a sensitivity of only 40–70%. This is due to low cellular yield and spreading of cells over a large area, which reduces the rate of detection of cells. Overcrowding, overlapping, cell loss and cellular changes due to processing are other important factors which reduce the sensitivity.

Multiple independent studies conducted on effusion cytology revealed that the cytospin and cell block methods are superior to conventional method in diagnosing the effusions. Due to lack of morphological details of the representative cells in the samples, conventional smears failed in making conclusive diagnosis. However, cytospin

Table 3: Diagnostic utility of cytospin in comparison to cell block

Category	Malignant (cell block)	Non – malignant (cell block)	Total
Malignant (Cytospin)	79 (True positive)	0 (False positive)	79
Non-malignant (Cytospin)	5 (False negative)	141 (True negative)	146
Total	84	141	225

Table 4: Comparison of results of cytospin and cell block with other studies

Result category	Present study (N=240)		Mulkalwar et al.[1] (N=170)		Sidhu et al.[3] (N=240)	
	Cytospin	Cell block	Cytospin	Cell block	Cytospin	Cell block
Inadequate	15 (6.2%)	15 (6.2%)	17 (10%)	17 (10%)	-	-
Non neoplastic	125 (52.1%)	124 (52.1%)	109 (64.2%)	109 (64.2%)	203 (84.5%)	206 (85.8%)
Suspicious of malignancy	21 (8.75%)	17 (7%)	10 (5.9%)	5 (2.9%)	10 (4.1%)	0 (0%)
Malignancy	79 (32.9%)	84 (35%)	34 (20%)	39 (22.9%)	27 (11.25%)	34 (14.1%)

Table 5: Comparison of sensitivity, specificity, PPV, and NPV with other studies

Parameter	Present study (N=240)	Nounechutuo et al.[5] (N=150)	Sidhu et al.[3] (N=240)
Year	2022	2020	2019
Sensitivity	94	75	87.5
Specificity	100	100	99.3
PPV	100	100	95.4
NPV	96.8	81.2	97.9

PPV: Positive predictive value, NPV: Negative predictive value

preparations allow the preservation of cellular details and reduce the overlapping of cells. Conventional cytology yields only suboptimal results as reported by Oygluso et al., with sensitivity of 44.5%, specificity of 95.7%, positive predictive value (PPV) of 98.7%, and negative predictive value (NPV) of 20%.[10] However, the present study using cytospin technique revealed a sensitivity of 94%, specificity of 100%, PPV of 100%, and NPV of 96.8% respectively.

Cytospin technique is better for concentrations of cells from fluid samples.[2] Cytospin technique also allows better preservation of cell morphology when compared to cell block method. Moreover, cytospin technique is less time consuming, relatively inexpensive, and easy and involves less technical manpower.[4] The drawback is that all cells, including blood cells and debris, are concentrated in a small area, which often tends to obscure any epithelial cells. Aggregation of mesothelial cells into clusters, rosettes, or acinar pattern can also confound the picture in cytospin smears.[11]

Cell block technique helps by processing of sediments, blood clots, or grossly visible tissue fragments from cytological specimens into the paraffin blocks that can be cut and stained by the same methods used for histopathology. This technique provides additional tissue architectural information. Cell block method can also be used for ancillary techniques such as immunohistochemistry and molecular studies. They can be useful for categorization of tumors that otherwise may not be possible from smears themselves.[2] The photomicrographs obtained through the cell block method provided better impression of malignancy than that depicted by cytospin smear method.[6] Cell block method is also useful when the cytological abnormalities in smear preparations are misleading, such as in distinguishing reactive mesothelial cells from malignant cells.[12] This is due to marked atypia of mesothelial cells caused by chemical, physical, immunological, and metabolic stimuli on the pleural membrane or due to subtle cytomorphological features of some malignancies such as well-differentiated adenocarcinomas.[13] Cell block method provides superior architectural patterns, morphological features between reactive mesothelial cells (Figure 1) and malignant cells and thereby increases the efficacy of cytodiagnosis.[14,15] Cell block technique was first introduced by Bahrenburg and it has been used for processing fluids which aid in diagnosis of benign and malignant lesions.[16] This technique is simple, safe, cost effective, and also reproducible in resource-limited rural areas.[7]

The advantages of the cell block procedure include:

1. Recognition of histological patterns of diseases that sometimes cannot be identified reliably in conventional smears.
2. Possible to study multiple sections by routine staining, special staining, and immunocytological procedures.
3. Less cellular dispersal, which permits easier microscopic observation than do traditional smears.
4. Less difficulty in identifying malignant cells in spite of background showing excess blood on microscopic observation.
5. Possibility of storing slides for retrospective studies. Storage of the cytological smear is a practical problem.[17,18]

Limitations of the study

A few samples showed degenerative changes in cytospin preparation and hence were not included in our study. Paucicellular samples were also excluded as they were nondiagnostic.

CONCLUSION

In the present study, cell block preparations undoubtedly aided the diagnosis of additional malignant cases and some with rare diagnosis. Even though cell block preparations provided superior architectural details and immunocytochemistry, it also had disadvantages such as increased turnaround time and loss of cellular material during processing. On the other hand, cytospin

preparations provide good cellular yield with reasonable preservation of cell morphology and was less time consuming. Considering the merits and demerits of both the techniques, it is imperative to use both cytospin to use both cytospin and cell block in the cytodiagnosis of peritoneal and pleural fluids.

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Authors Contribution:
LMJ- Concept and design of the study, manuscript preparation, result analysis and interpretation. **SS-** concept and design of study, guiding the progress of the study, reviewed result analysis and interpretation. **DS-**Concept and design of the study, Reviewed the literature, result analysis and interpretation, manuscript preparation of the article. **SS-** Concept and design of the study, Reviewed statistical analysis and interpretation, Guidance and support..

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