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COMPARISON OF EFFICACY OF CELL BLOCK VERSUS CONVENTIONAL SMEAR STUDY IN EXUDATIVE FLUIDS - AND ITS ROLE IN DIAGNOSTIC CYTOLOGY

Swati Singh[1], Mayurika Tyagi[2], Adreena Mittal[3], Geetanjali Yadav[4*]

[1]Professor, Pathology, Santosh Medical College, Santosh deemed to be university, Ghaziabad, Uttar Pradesh, India
[2]Professor & HOD, IHBT, Santosh Medical College, Santosh deemed to be university, Ghaziabad, Uttar Pradesh, India
[3]Professor & HOD, Pathology, Santosh Medical College, Santosh deemed to be university, Ghaziabad, Uttar Pradesh, India
[4*]JR3, Pathology, Santosh Medical College, Santosh deemed to be university, Ghaziabad, Uttar Pradesh, India Mail id - y.geetanjali030@gmail.com

***Corresponding Author:** Geetanjali Yadav
[*]JR3, Pathology, Santosh Medical College, Santosh deemed to be university, Ghaziabad, Uttar Pradesh, India Mail id - y.geetanjali030@gmail.com

Abstract

Introduction: Cell blocks prepared from residual tissue fluids can be useful adjuncts to smears for establishing a more definitive cytopathologic diagnosis. They can be particularly useful for categorization of tumors that otherwise may not be possible from smears themselves .CB technique increases the diagnostic accuracy due to increased cellularity, preservation of tissue architecture and feasibility of performing ancillary techniques like immunohistochemistry (IHC).

Aims and Objectives: To compare the cytological features of pleural and peritoneal exudative fluids by conventional smear (CS) method and cell block (CB) method and to assess the utility of a combined approach for cytodiagnosis of these effusions.

Materials and Methods: The study was done on all exudative fluid samples received in the Pathology lab for cytological examination .All fluids were divided into two equal parts: one part was subjected to CS technique, while the other part was subjected to Plasma thromboplastin CB technique and the sections stained by Hematoxylin and Eosin. IHC was performed whenever required.

Results: 128 pleural and peritoneal fluid samples were processed. 07(5.4 %) cases were positive for malignancy on conventional smear, whereas on CB 18 (14 %) cases were diagnosed as malignancy. The additional yield of malignancy was 9 % more by the CB method.

Conclusions Our study showed that the CB method yielded better results in diagnosis of malignancy. Therefore, this technique could be considered as a useful adjuvant in evaluating fluid cytology and final cytodiagnosis, along with the routine CS method.

Keywords : Cellblock, Conventional smear, Immunohistochemistry, fluid cytology

INTRODUCTION

The cytological examination of body fluids is of paramount importance not only for diagnosis but also for the staging, prognosis and further management of the patient [(1)]. The involvement of the

serous cavities by malignant neoplasms has important therapeutic and prognostic implications. The most common reason to submit an effusion fluid to cytopathology is to determine whether or not it contains malignant cells [2]. Preparation of conventional smears (CSs) of effusions is a fairly simpler procedure as compared to preparation of paraffin sections, but it has a low sensitivity for detecting malignancy. This is attributed to lack of tissue architecture, overcrowding and overlapping of cells, and artifacts due to suboptimal processing and delay in processing.[3,4] Cytological examination of fluids by means of smears, however carefully prepared also leaves behind a large residue that is not further investigated but that might contain valuable diagnostic material.

This residual material can be evaluated in a simple and expedient fashion by treating it as a cell block, embedded in paraffin, and examined in addition to the routine smears.[5,6] The additional benefits of CB technique are cell enrichment, lesser cellular dispersal, preservation of specific tissue architecture, better morphological details, the familiarity of the Hematoxylin and Eosin (H and E) stain and feasibility of performing ancillary studies i.e., special histochemical stains and immunohistochemistry (IHC) [3,7,8]. Although CB technique has been known for nearly a century, there have been few reports and a limited number of samples involving the direct comparison of CS and CB on consecutive patients for diagnosis of exudative effusion. The aim of this study was to compare the diagnostic yields of CS, CB technique and the combination of both, regardless of the etiology of effusion and also performing IHC in confirming the diagnosis of malignancy where the CS were suspicious and identifying the primary site of malignancy .

MATERIAL AND METHODS

The present study was conducted in the Department of Pathology of a tertiary hospital over a period of 12 months on pleural and ascitic fluid samples received in the Pathology lab for cytological examination.

Inclusion criteria

All pleural and ascitic fluid which were exudative in nature and having protein level of >3.0 gm/dl.

Exclusion criteria

1. All other fluids except pleural and ascitic
2. Fluids which were transudative in nature, i.e., having protein level of <3gm/dl.

Relevant information regarding age, sex, clinical and radiological findings was obtained from the patient records.

Processing of fluids

All fluids were divided into two equal parts. One part was kept for conventional cytology smear preparation (centrifuged smear– CS) while the other part was used for CB preparation. Half of the fluid, about 5 ml was centrifuged, supernatant fluid discarded, smears prepared and stained with May-Grunewald-Giemsa and or Papanicolaou stain which was used wherever necessary. The remaining sample was subjected to centrifugation at a rate of 1500 rpm. The supernatant fluid was discarded and the sediment or the cell button, thus obtained was fixed for 24hrs in 10% formal-alcohol (combination of ethyl alcohol and formalin) and then processed in a histokinette like a routine histopathology sample. The sections were stained with H&E.

The cytological smears and block sections were examined separately for cellularity, architectural patterns and morphology (cytoplasmic and nuclear details) to render a cytological diagnosis for each case, and the findings were compared. A panel of immunomarkers were utilized in doubtful cases to distinguish atypical mesothelial cells from metastatic malignancies and also to categorize the type and the primary site of malignancy in a few select cases to complete the work up.

RESULT

In the present study, a total of 128 serous fluid samples were processed both for CS and CB preparation. Pleural fluid constituted 78 (60.9%) of cases while peritoneal fluid constituted the remaining 50 (39.1%) cases. The age of the patients ranged from 22 to 80 years with a mean of 54 years. The peak age was in the 41-50 year age group, accounting for 29.8 % of cases in the study. There was a slight female preponderance seen in the study. 54.6% of patients included in the study were females and Female to male ratio was 1.2:1 in the study. A cytological diagnosis was rendered for each case in the study and each individual slide was objectively analyzed for cellularity, arrangement (acini, papillae, cell balls, and proliferation spheres), cytoplasmic, and nuclear details, using a three tier classification system. Results were grouped into benign, suspicious for malignancy and malignant category. The results obtained on conventional cytology and cell block were than compared (Table 1).

Table 1: Comparison of cytological diagnosis on conventional smear and cell block study

No	Diagnostic Category	Conventional Smear (CS)n %	Cell block (CB)n %
1	Benign	106 (82.9)	107 (83.6)
2	Suspicious for Malignancy	15 (11.7)	03 (2.4)
3	Malignant	07 (5.4)	18 (14.0)
	Total	128 (100)	128 (100)

No case was found to be diagnostically unsuitable on CS or CB study.

There were 90 (70.3%) cases which showed mild cellularity on cytological smear whereas moderate and marked cellularity were seen in 28(21.8%) and 10 (7.9%) of cases, respectively.

Similarly, there were 65 (50.7%) mildly cellular CBs while moderately and markedly cellular CBs were seen in 48 (37.5%) and 15 (11.8%) cases, respectively. Architectural patterns, such as cell balls, sheets, cell clusters, glands and papillae were better observed in CBs as compared to CS.

One hundred and six fluid specimens were categorized as benign effusion by CS method106 (82.9%). The majority of cases were due to tuberculosis. Other diseases causing reactive benign effusions were congestive heart failure cases, cirrhosis of liver cases, bacterial infection cases.

15 (11.7%) fluids samples were labelled as suspicious for malignancy on conventional smear cytology while 07(5.4%) fluid specimens were categorized as malignant effusion by CS method alone. (Fig 1)

By the CB method, apart from the confirmed malignant cases on cytology alone additional 11 cases were detected as malignant, that is almost 9% more diagnostic yield for malignancy with addition of cell block with conventional cytology. (Fig 2)

The cell block technique not only increased the yield of positive results, but also helped to demonstrate better architectural patterns which could be of great help in approaching the correct diagnosis of the primary site. It was possible to ascertain the primary site of malignancy in 92 % of the cases with the help of cell blocks and smears.

The cell block technique has an added advantage that multiple sections of the same material can be obtained for special stains and immunohistochemistry. Application of immunohistochemistry markers was beneficial in our study especially in cases where morphology alone was inconclusive for diagnosis either because of scant material or ill preserved architecture. Calretinin, CK 5/6, WT1 were useful positive tissue markers for mesothelioma and CEA, the best negative markers for distinguishing between epithelioid mesotheliomas and adenocarcinoma. (Fig 3)

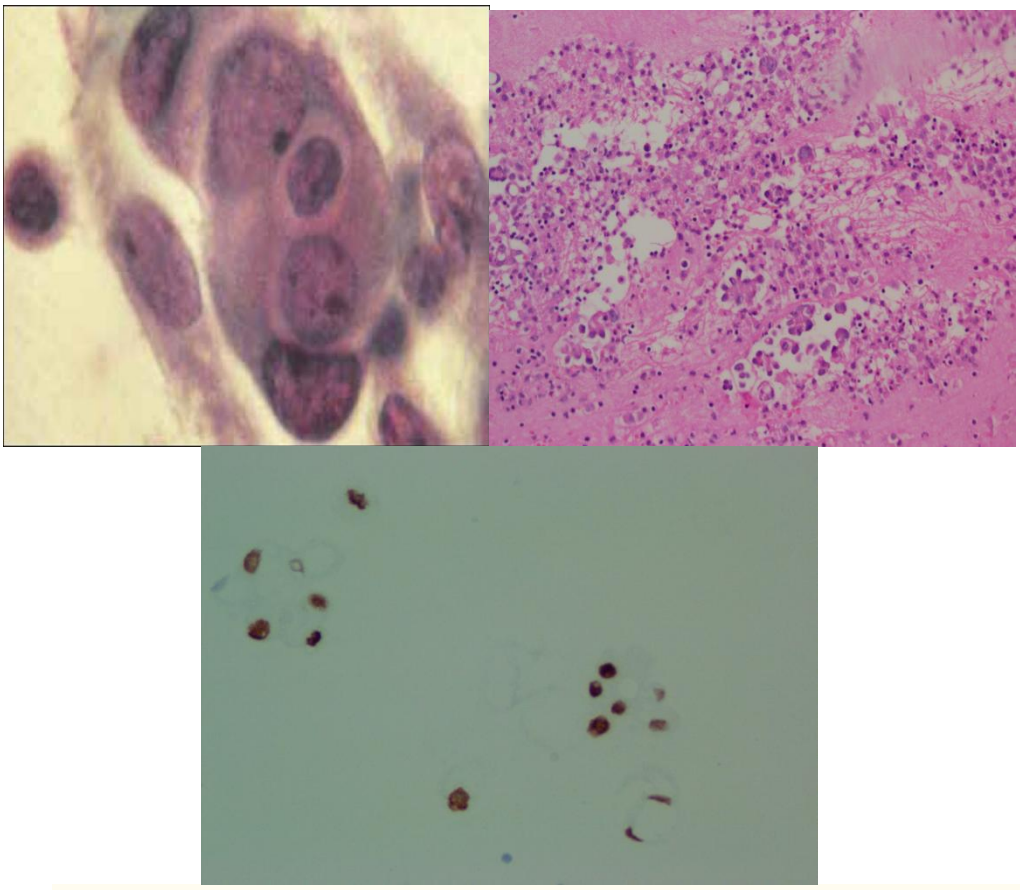

Fig 1- Ascitic fluid smear from a case of ovarian serous carcinoma (MGG, ×500)
Fig 2- Cell Block preparation showing Features of Adenocarcinoma (40x)
Fig 3- Cell block positive for Calretinin (40x)

Discussion

The current study studied 128 specimens among which pleural fluid was the commonest (60.9 %) followed by ascitic fluid (39.1%). This is in concordance with other studies who also reported pleural fluid as the commonest of all effusions. In their study of effusion, Luse and Reaga [9] reported that the maximum number of cases of effusion were of pleural effusion, followed by peritoneal fluid.
The cell block method is the oldest method of processing cytological material, described by Mandlebaum in 1900 for studying exudate. [10] 10% alcohol-formalin is used for fixation and by the action of formalin, the proteins are cross-linked and a gel is formed which can't be dissolved in any material used for processing. [11]

In the CS method, reactive mesothelial cells, paucity of representative cells, abundance of inflammatory cells obscuring the morphology of atypical cells and subtle morphological features of some malignant neoplasms contribute to the difficulties which are faced in making a diagnosis previously. The CBs which are prepared from the residual fluids were found to be particularly useful for the identification of tumors that cause diagnostic difficulties in CS.
In the present study of 128 cases of cell block the predominant lesion detected in the various fluids was benign inflammatory effusion while malignancy was detected in 18 (14%) cases. By the CB method, apart from the confirmed malignant cases on cytology alone, additional 11 cases were detected as malignant, that is almost 9% more diagnostic yield for malignancy. These 11 fluid specimens were reported as suspicious for malignancy by CS.
In the present study, simultaneous use of the cell block technique and smear examination increased diagnostic accuracy by 9.0%, consistent with the 12% increase reported by. Richardson*et al*. [12]

Table 2 Comparison of the diagnostic yield of smear versus cell block in various studies

	Archana et al [13]	Sujathan et al [14]	Present study
Total cases	150	85	128
Positive for malignancy on smear	29	19	7
Positive for malignancy on cell block	39	21	18

Out of 150 cases studied by Archana et al,[13] 39 (26%) were positive for malignancy by cell block method, while by routine method only 29 samples were reported as positive for malignant cells. Thus it was found that there was significant difference between the results obtained by direct smears method and cell block method.
Supplementing CB was the use of a case based panel of antibodies for IHC used in the study. This added expression of markers by IHC was instrumental in objectively defining the cell of origin of the malignant cell, which enabled us to clearly ascertain the primary malignancy and also in cases where morphology alone was insufficient for a definitive diagnosis.

The present study also emphasizes that it is advisable to study paraffin sections by using the cell block method even for smears which are negative on l smear examination only .Similar conclusions were drawn by Foord and Wetmore[15] who conducted cellular studies of effusions by using smears and paraffin sections. They preferred to study paraffin sections before giving the final diagnosis because it was more accurate and as it was easier to demonstrate cellular relationships with the cell block technique.

Conclusion

In summary, cell block preparation is a powerful tool that complements conventional cytology, offering enhanced diagnostic capabilities, particularly in malignancy detection. Combining both methods can optimize the evaluation of exudative fluids.
We conclude that the cell block technique when used as an adjuvant to routine smear examination has increased the diagnostic yield because of better preservation of the architectural pattern, particularly in cases where there is a diagnostic dilemma between the malignancy and reactive changes. Immunohistochemistry also gives better results on the tissue in the cell block than cytological smears which will be helpful to arrive at the accurate diagnosis.

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