

Comparative Study of Cell Block Versus Centrifuged Smear Examination from Aspirates of Body Cavities - A Cross Sectional Study in a Tertiary Care Hospital

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Abstract: *Background:* Evaluation of effusion fluid cytology has become integral part of management in suspected malignant cases. Conventional smear (CS) is relatively simple, but it becomes challenging to differentiate in cases of reactive mesothelial cells and malignant mesothelial cells. Hence, Cell block (CB) preparation is gaining popularity for management. *Objectives:* To depict a comparison between conventional cytology smear and cell block preparation of all types of fluid. *Material and methods:* All the fluid samples received in the department of pathology, were divided into two parts, one part was stained for conventional smears and the second part was centrifuged and Bouin's fixative was added and cell block was made and stained with papanicolaou (PAP) stain and Haematoxylin - Eosin stain (H&E). Diagnosis obtained on conventional smears and cell block were statically analyzed. *Results:* Out of total 30 fluid samples, 17 were pleural fluid, 8 were Ascitic fluid and 5 cases were peritoneal fluid. 1 fluid sample was unsuitable, 28 fluid sample were adequate, 1 fluid sample was adequate and suspicious for evaluation, 19 cases were non - neoplastic, 2 were suspicious for malignancy, 9 cases were positive for malignancy. *Conclusion:* Cell block is much superior to conventional cytology smears for diagnosis of malignant effusions and hence Cell block should be used in adjunct to conventional smears in difficult or suspected malignant cases in routine practices.

Keywords: Cell block preparation, Body Fluids, Effusion cytology, Conventional Smears.

1. Introduction

Cell block technique was first described by Bahrenberg in 1896.¹ This is an old method for evaluation of body cavity fluids. The cell block technique uses the method of retrieval of cells/small tissue fragments from any body fluid including ascitic fluid, pleural fluid, bronchial wash.² Conventional cytology smears have got some drawbacks due to overcrowding of cells and cell loss due to less cellularity. Cell block can be prepared by methods using plasmathrombin/agar method/Bouin's fixative method.³ Cell button formed is formalin fixed and processed routinely like histopathological specimens.⁴ The advantage of cell block is the ability to make multiple sections and the architecture of the tissue were preserved in cell block. Immunohistochemistry is an effective tool, that can be used on cell block to distinguish and sub classify the malignancies.⁵

Objectives:

To depict a comparison between conventional cytology smear 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 specimen of fluid cytology in addition to conventional smears.

2. Materials and methods

It is a prospective study, conducted in department of pathology, where 30 samples of body fluids were collected and conventional smears and cell block preparation were

made out of all types of fluid specimen received in the cytology section in the pathology department of Sree Mookambika institute of medical sciences, Kulasekharam (2018 - 2020).

The clinical details of patient like name, age, sex and diagnosis were recorded.

After reporting the conventional cytological smear, the representative received samples were processed for cell block preparations immediately.

Some samples were stored in refrigerator at 4 degree and processed on the next day.

Whenever a fluid specimen is received, it was divided into two parts and transferred into two test tubes. One sample is centrifuged at (REMI CENTRIFUGE) at 2500 rpm x 10 minutes and supernatant is discarded, then centrifuged deposit/sediment is poured into glass slides and made smears.

The smears were fixed in 80 - 95% isopropyl alcohol for 20 minutes and then stained with Pap smear and H&E stain.

Remaining part of the sample were used for cell block preparation.

In case of lesser quantity of body fluids received, then whole fluid is taken for centrifuge at 2500 rpm x 10 minutes, the supernatant obtained is discarded and the sediment received

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is taken 2 - 3 drops for conventional smears and rest of the sediment is added with bouin's fixative and centrifuged and cell block preparation is made.

**Type of fixative used for cell block preparation:
BOUIN'S FIXATIVE**

Composition of Bouin's Fixative:

- Saturated Picric Acid solution - 75 ml.
- 10% Formaldehyde - 25 ml.
- Acetic Acid - 5 ml.

Method of cell block preparation:

Centrifuge the fluid and discard the supernatant material and take the sediment for conventional smear preparation and other half for cell block preparation.

For cell block preparation, take the conical plastic centrifuge tube again and add the fluid and centrifuge at 2500 rpm x 10 minutes.

Remove the supernatant and to the sediment add equal amount of Bouin's fixative, then centrifuge the sample again, the supernatant obtained was discarded.

The remaining portion of the sediment is kept in filter paper and added to 10% formalin and processed for routine H& E staining.

3. Results

Out of 30 cases, 17 cases were pleural fluid, 5 cases were peritoneal fluid and 8 cases were ascitic fluid which were analyzed. Total males - 14 cases and females - 16 cases. Out of 17 patients having pleural effusion, 10 were male and 7 were female, out of 5 cases of peritoneal effusions, 4 were female and 1 were male, out of 8 cases of Ascites 5 were female and 3 were male.

The findings regarding diagnosis by conventional smears and cell block preparation in pleural, peritoneal and ascitic fluid were tabulated in table:

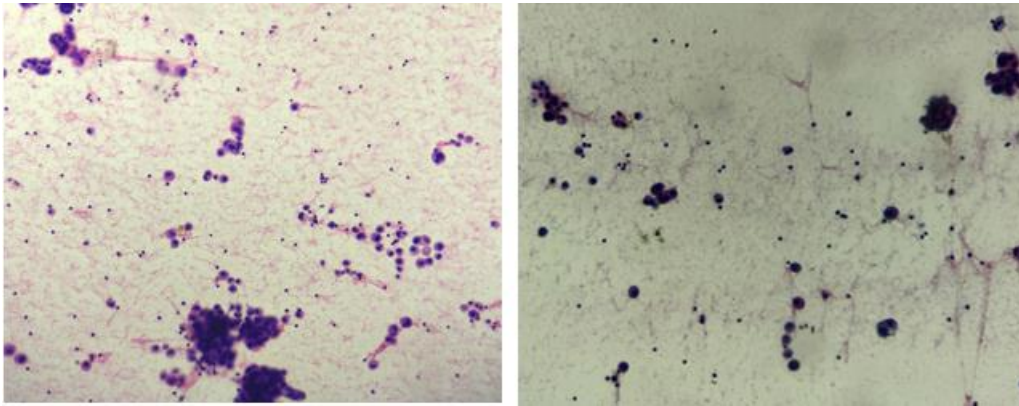


Figure 1a and 1b: Conventional smear on pleural fluid - positive for malignancy - Suggestive of Adenocarcinoma

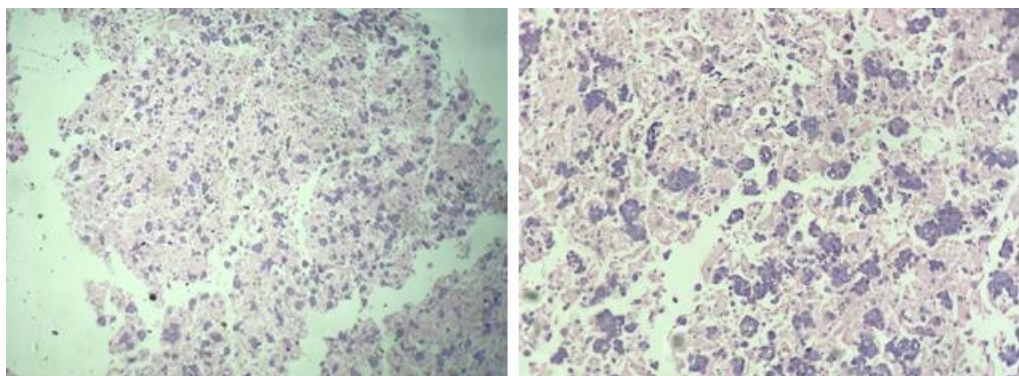


Figure 2a and 2b: Cell block on pleural fluid - Positive for malignancy – Suggestive of Adenocarcinoma

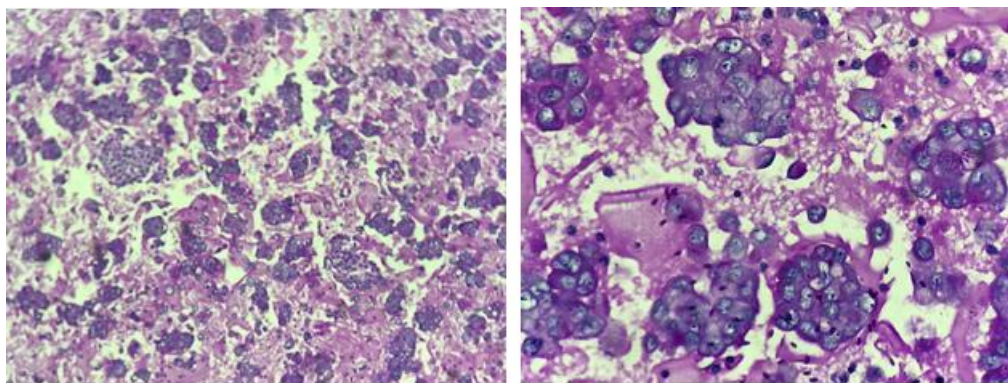


Figure 3a and 3b: PAS - D on Cell block showing positive for malignant cells - suggestive of Adenocarcinoma

Table 1: Study population of conventional smear and cell block in pleural fluid cytology

Study population of CS and CB	
Male	14
Females	16
Total	30

Table 2: Different types of body fluids studied in conventional smear and cell block preparation:

Types of Fluid	Total number of Cases	Conventional Smear		Cell Block	
		non – neoplastic	Neoplastic	non – neoplastic	neoplastic
Pleural fluid	17	11	6	7	10
Ascitic fluid	8	4	4	4	4
Peritoneal fluid	5	5	0	5	0

Table 3: Diagnostic criteria of fluid cytology in conventional smear and cell block preparation:

Diagnostic category of CS and CB	CS method	CB method
Non - neoplastic	19	19
Suspicious for malignancy	2	2
Positive for malignancy	9	9

Table 4: Adequacy of fluid cytology in conventional smear and cell block preparation:

Adequacy of CS and CB	CS	CB
Unsuitable	1	1
Adequate	28	28
Adequate and suspicious	1	1
Total	30	30

4. Discussion

The effusion fluid cytology for conventional smears and cell block preparation has increasingly gained acceptance. It not only helps for the diagnosis of malignant lesions, but also important for staging and prognosis.

Bodele et al⁶ studied that increased cellularity by MCB better morphological details and preservation of architectural pattern like in dimensional clusters, cell balls, acinar pattern compared to cell conventional smears.

Sujathan K et al⁷ studied that the CB technique, which is one among the oldest methods of processing the cytological materials for microscopy. The study also stated that CB preparations are routinely used in some cytology laboratories in developed countries, particularly for ICH, where it is often used in difficult cases. But the cell block method, we describe is very simple, rapid and cost effective, since no additional materials are required, since the use of CB increased the

diagnostic yield of malignancy from 19 to 21 samples in their study.

The study conducted by Dekker et al⁸ stated that the CB puts both features in their pros. The nucleoli do not appear as prominent as in the smear and the pseudoacinar and acinar structure can be better appreciated when present. The study also stated that CB is a valuable tool in the evaluation of well differentiated adenocarcinoma, such as in tumors of breast, lung or GIT.

Another advantage of cell block is the concentration of cellular material in one small area that can be evaluated at a glance with all cells lying in the same focal plane of the microscope.

In this study conducted by Shobha SN et al⁹, 95% alcohol - formalin was used as a fixative for modified cell block preparation. By this method, better cellularity was obtained compared to conventional smears as formalin minimized the cell loss by forming protein cross links.

Similar fixative was used in study conducted by Bodele et al, ⁶ where 100 samples of pleural fluid was received and effusion was found to be less common before 20 years of age and the highest number seen in the fifth decade and effusion was more common in males compared to females. In our present study females (16) are more than males (15).

In the present study, diagnostic yield for malignancy was by was increased by CB, out of 30 cases, the cases which are suspicious for malignancy on conventional smear, was confirmed as malignant in cell block, so additional yield was obtained on cell block.

Study conducted by Nathan NA et al¹⁰ stated that both the absolute alcohol and formalin solution techniques are used for general purpose grade reagents that are used routinely in cytology and histology laboratories, in their study they did not use 95% ethanol, instead of absolute alcohol, in this method 13 hr. processing schedule routinely used similar to small biopsy tissue processing. This correlated well with the present study. CB has advantage over CS, improper smear fixation and staining techniques in CS can cause cell overlapping or overcrowding, cell loss, artifacts and poor background staining, while these are less frequent in CB. Cellularity is higher by cell block compared with conventional smears and is concentrated at one end that can be evaluated at a glance with all cell lying in same focal plane of microscope. 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.¹¹ The study conducted by Niveditha et al¹² studied 220 samples of fluids from different sites of which majority were pleural fluid (34%), followed by peritoneal fluid samples (29.1%). 86.8% cases - benign, 1.8% were suspicious for malignancy, 8.2% positive for malignancy.

In the present study 30 fluid samples were taken for study, ascitic fluid 8, pleural fluid 17, peritoneal fluid 5 cases, in the present study 19 benign cases, suspicious for malignancy - 2 cases, 9 positive for malignancy cases. In the present study 95% alcohol fixed smears were used. Konikov et al (1966)¹³ had used H & E stained cell block technique.

Study conducted by Barui et al¹⁴ stated that one of the most common problems in conventional smear cytology is to distinguish reactive mesothelial cells from metastatic deposits, especially adenocarcinoma. Although the preparation of conventional smear is a much simpler and cost effective procedure than that of cell block, it has few limitations like lack of tissue architecture, leaving behind useful material, moreover, conventional cytological examination of effusion fluid has sensitivity of 40 - 70% to detect the presence of malignant diseases due to overcrowding of cells and cell loss and different laboratory processing methods.

5. Conclusion

- The conventional smear and cell block preparation slides were evaluated on the basis of cellularity, architectural morphology and diagnosis.
- Cell block preparation was much superior in yielding the diagnosis of malignancies compared to conventional cytology smears.
- Cell block preparation has superior role than that of conventional smear to differentiate reactive mesothelial cells from metastatic adenocarcinoma.
- Cell block preparation can also be used to perform Immunohistochemical stains (IHC), where IHC will be necessary to substantiate the diagnosis in case of malignancies.
- Hence, Cell block can be used as a complementary investigation in addition to conventional cytology smears.

Funding:

- Self

Conflicts of interest:

- No

6. Future Recommendations

- Studies in large numbers are essential.
- Faster techniques like microwave processing could be attempted in cell block preparation.

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