ANALYSIS OF CELL BLOCK VS. CONVENTIONAL SMEAR IN FLUID CYTOLOGY
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ABSTRACT: BACKGROUND: The cytological examination of aspirates of serous effusions is a routinely accepted, simple, safe and minimally invasive technique. Diagnosis in this investigation, especially in malignant effusions, helps in staging, prognosis and management of the patients. AIMS: To assess the utility and sensitivity of cell block method over conventional smear technique in cytodiagnosis of the serous effusions. METHODS: A total of 72 fluid specimens were subjected to simultaneous processing by conventional smear and cell block technique. Each fluid specimen was divided into two equal parts and results compared for cellularity, cell architecture, cytoplasmic and nuclear features. Cell blocks were prepared using modified cell block technique using alcohol formalin fixative. RESULTS: The utility of cell block technique in diagnosing malignant effusions is highly significant as compared to the conventional smear technique. Also, the technique using alcohol formalin fixative is simple, safe and these chemicals are routinely used in laboratory. CONCLUSION: Cell block technique is superior to conventional smear technique, especially for malignant effusions. It gives more information about the architectural arrangement and the likely source of primary. More important is that diagnostic material in cell blocks is available for special studies for. Immunohistochemistry which can further supplement our knowledge about the primary source of metastasis. KEYWORDS: Cell Block, Conventional Smear, Effusions.

INTRODUCTION: The Cytological examination of serous effusions has been a routinely done investigation in cytology. A positive diagnosis especially for malignant cells, is always taken as definitive diagnosis and assists the clinicians in deciding the further course of action and treatment of the patient. It is a relatively simple and noninvasive technique, which helps in coming to a conclusion about etiology of effusion as inflammatory, benign or malignant.(1) Many a times, it becomes difficult to accurately identify the cellularity in conventional smears as highly reactive mesothelial cells may simulate malignant cells. The cause for this practical problem is mainly bland morphology of cells, cellular overcrowding or overlapping, artefactual changes because of delay in processing, variations in laboratory preparatory techniques and useful material left behind in centrifuge tube in conventional smear method.

The residual material, many a times contains valuable diagnostic material and this can be utilised to increase the cell yield by one of the methods of evaluation of body cavity fluids i.e., cell block technique. Cell block is embedded in paraffin and examined for types of cells in fluid, in adjunct to conventional smears.(2) Cell block technique increases cell yield with increase in sensitivity of the test and decrease in number of false positive results. A modified cell block technique using alcohol
formalin fixative, followed by routine tissue processing has been used, which offers better preservation of architectural details of cells and this can be used for special stains and immunocytochemistry. This method is a simple and inexpensive technique and uses routine laboratory chemicals.\(^{(3)}\)

In our study cell yield and cytodiagnosis obtained by cell block technique has been compared with conventional smear technique. We have come to the conclusion that cell block technique improves the diagnostic accuracy in fluid cytology.\(^{(1)}\)

**MATERIALS AND METHODS:** The present study was carried out on 72 samples of serous effusions received in cytology section of department of pathology. The fluid samples received were processed by conventional smear and cell block technique simultaneously for comparative study of the results.

Fluid samples received were divided into two test tubes (5 ml each Approx).

**The Conventional Smear Technique:** The 5 millilitre sample was centrifuged at 2500 rpm for 15 minutes. Two or more thin smears were prepared from the sediment. At least one smear was prepared after air drying and stained with the May-Grunewald-Giemsa stain. The other smear was immediately fixed in 95% alcohol and stained with the Papanicolaou stain.

**The Cell Block Technique:** Second test tube with 5ml sample was subjected to fixation for one hour by mixing it with 5ml of 10% alcohol–formalin. This 10 ml fluid was centrifuged at 2500 rpm for 15 minutes after one hour. 10% alcohol–formalin was again added to the sediment and kept overnight. Next day, the cell button was scooped out on to a filter paper, processed like routine biopsy specimens, paraffin embedding done, 4–6 μ thickness sections cut and stained with routine hematoxylin and eosin stain.

The samples were studied in detail simultaneously with cell block and conventional smear techniques. The samples were then categorized as benign or negative for malignancy, suspicious for malignancy, or positive for malignancy the morphological criteria that were considered included the cell yield, the architectural arrangement of the cells and details of cytoplasmic and nuclear features. All these criteria were put together and they were used for the categorization of the sample. A comparison was done for observations by cell block and conventional smear method.

**RESULTS:** 72 samples were screened using conventional smear & cell block techniques simultaneously. The age of the patients range from 2-80 years, with maximum no of patients in the age group of 60-70 years. The samples of female patients (43) outnumbered the male patient’s samples.

The yield of cells and the details of architectural pattern and detailed information about cellular as well as nuclear features, were better appreciated in the cell block as against the conventional smear method. It has been observed that CS show individually dispersed cells, clusters, papillary fragments and acinar formations & signet ring cells or keratinised cells in malignant fluid effusions, but the appreciation of architectural pattern of the malignant cytology,
such as, three dimensional clusters, cell balls, sheets, cellular as well as nuclear pleomorphism, nuclear hyperchromatism, irregularity of the nuclear contours, type of chromatin, prominence of nucleoli, atypical mitotic figures and features of differentiation such as intracellular secretions, signet ring cells & evidence of keratinisation is much better in cell blocks. (FIG. 1 & FIG. 2)

After the analysis of fluid samples, including ascitic & pleural fluids, results were categorised as benign (Both inflammatory and negative for malignancy), suspicious for malignancy and malignant effusions (Table 3). Inflammatory effusions showed prominence of neutrophils or lymphocytes in the effusions, depending on the aetiology, along with presence of benign reactive mesothelial cells. 4 samples were purulent on physical examination and the microscopy was also consistent with pus, with presence of neutrophil rich cellularity. It was observed that the cell blocks of this case show numerous inflammatory cells, rich in neutrophils and few reactive mesothelial cells.4 cases reported as benign on CS, showed obscured morphology of cells on cell block by haemorrhagic material.

Out of 4 cases reported suspicious of malignant cells on CS, 3 were reported as malignant on CB. One of the case was reported as ascitic fluid positive for metastatic adenocarcinoma and on complete work up was diagnosed as carcinoma stomach & the second positive case showed presence of atypical keratinised cells in pleural fluid The female patient had presented with complaints of fever, cough and weight loss, & on clinical work up was diagnosed as carcinoma lung. The third case positive for malignant cells on ascitic fluid was a case of colorectal carcinoma with relapse.

The malignant effusions were common in females and the most common primary identified was from ovary (n=5). Out of all the malignant effusions on CS & CB, 5 primaries were from ovary, 2 breast, 2 stomach, 2 colorectal, 2 lung & 1 metastatic deposits in liver with unknown primary. The utility of cell block in cytodiagnosis of malignant effusions has been found to be highly significant as compared to conventional smears in our study.

Chi square test was used to analyse the benign and malignant lesions by CS & CB method In which the p value was found to be highly significant. (Table 1). CB method is highly significant in the detection of malignancy (Table 2).

**DISCUSSION:** The importance of cytological examination of the serous effusions is a routinely done procedure in cytology laboratories of department of pathology everywhere. It is of considerable importance in various benign conditions like hepatic cirrhosis, pleurisy, pulmonary infarcts & in suspected malignant effusions as well as in staging in patients with known primary malignancy. Examination of fluid cytology is of paramount importance & has the diagnostic, therapeutic & prognostic implications. The presence of malignant cells in the pleural or ascitic fluid are almost always indicative of metastasis and advanced stage of malignancy as the primary malignancies of the mesothelial lining are very rare. This test is also an important investigations a diagnostic and prognostic indicator in oncology practice. This investigation helps to detect unsuspected cancers & the metastasis from known primary.

Beale introduced the paraffin-block method for serous effusions in 1895.[4] In 1896, Bahrenberg first described the cell block technique and it was commonly used after Mandlebaum reported the finding of actinomyces in a cell block.[5]
In the CS method, reactive mesothelial cells, an abundance of inflammatory cells and a paucity of representative cells contribute to the considerable difficulties which are faced in making conclusive diagnosis. Another limitation of the conventional cytological examination of effusions is that it has a sensitivity of only 40–70% for detecting the presence of malignant diseases, due to the overcrowding of the cells, cell loss and also due to the different laboratory processing methods. The difficulty is either secondary to the marked atypia of the mesothelial cells which is caused by the microbiological, chemical, physical, immunological, or the metabolic insults to the serous membranes or due to the subtle cytomorphological features of some malignant neoplasms. The problem may become compounded due to the artifacts which are caused by poor fixation, preparation, or staining techniques. For this reason, in this study, an attempt was made to prepare and to analyze both the CS and the CB which were prepared by using 10% alcohol- formalin as a fixative, from the same specimen.

Since the introduction of the cell block technique by Behrenburg nearly a century ago, it has been routinely used for processing fluid samples. The cell blocks prepared from residual fluid samples can be used in addition to conventional smear technique for reaching a definite cytological diagnosis. This technique is simple to perform and no special expertise is required for handling the specimens.

In 1928, Zemansky concluded that the cell block technique was far superior to conventional smear technique. CB has long been a useful complementary method for examination of cytologic material. Since large amount of diagnostic material is still left behind after smear preparation and it receives no attention. This can be used in CB preparation thus increasing the sensitivity of detecting malignancy and also has the ability to reduce false positive interpretation. Cases that are suspicious or equivocal on the smear can be diagnosed definitively on cell block. The examination of cell block preparation along with smear yield two differing, complimentary views of the same cell population thus increasing the sensitivity and decreasing false positive results.

In earlier studies, a wide range of histologic fixatives have been used for cell blocks, like buffered formalin, neutral buffered formalin solution, Bouin’s solution, picric acid fixative, Carnoy fixative and ethanol. All the above mentioned chemicals are inconvenient for routine use and are time consuming and they require special attention and care too. In the present study, alcohol and formalin were used as fixative. Both these are general purpose grade reagents that are used routinely in cytology and histopathology laboratories.

Nathan N A et al (2002) had developed this modified cell block technique using an improvised ethanol formalin fixative (Nathan alcohol formalin Substitute) followed by a simple paraffin processing and showed its increased efficacy in diagnostic cytology. A definitive cytopathological diagnosis of aspirates of serous effusions esp the malignant ones, is an important diagnostic indicator in clinical practice. A positive diagnosis for malignant cell in effusions changes the stage and prognosis of the patient and helps in deciding the treatment protocol. Even a result which says “negative for malignancy” is equally important. This is the reason why we need a more definitive method than conventional smear to be sure of our results. The positive malignant pleural effusion is a common complication of lung, breast and gastric carcinomas and malignant ascitic effusions are common in colon and ovary. The reactive
mesothelial cells, as seen in hepatic cirrhosis, pleurisy, may also show reactive changes as cytomegaly, nucleomegaly, multinucleation. The problem may further be compounded by poor fixation artefacts and other artefacts due to preparatory and staining techniques. The advantage of conventional smear technique is that it is simpler procedure but with the limitation that the storage of these slides is a practical problem and that there is lower cell yield and lack of tissue architecture in most of the cases. Furthermore, cell block method has also become simple, safe and convenient. More important is that diagnostic material in cell blocks is available for special studies for. Immunohistochemistry which can further supplement our knowledge about the primary source of metastasis. The paraffin block effectively puts the morphological features in their proper perspective, with less of cell dispersal and advantage of storing slides and material for future use. The main disadvantage is the delay in diagnosis as well as risk of losing material during processing.

Moreover routine cell block making by using by adding agar or plasma thrombin are not cost effective methods, but the cell block technique using 10% alcohol formalin as a fixative is simple, inexpensive and does not need any special expertise. Also, the use of alcohol based fixative provides a better preservation of antigenicity and architecture and morphology as compared to conventional technique. Various studies document that an additional diagnostic yield for malignancy was added when conventional smear was supplemented by cell block technique Koss (2006) stated that cell block technique be used for processing residual material left after processing using conventional methods.(4)

Since then many more studies have been done to compare the results of conventional smears with cell block technique. This has been proven time and again that cell block technique is simple, cost effective and safe and reproducible even in resource limited settings like ours. The cell block technique provides best interpretation of cell morphology. Positive malignant effusions indicate advanced stage of cancers. Positive malignant cells in pleural fluid are seen in primaries such as lung cancer, breast cancer and gastric cancer, whereas malignant ascitic effusions indicate primary from ovary, colon, liver, pancreas. Hence, positive malignant effusions indicate widespread metastasis and advanced stage of malignancies and help in staging, prognosis and management of the patients. Henceforth, the cytological examination of serous effusions is of paramount importance in diagnostic, therapeutic and prognostic implications.(10,11)

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| Technique        | Malignancy (+) | Malignancy (-) | Total |
|------------------|----------------|----------------|-------|
| Cell block       | 14             | 16             | 30    |
| Conventional Smear | 06             | 24             | 30    |

Table 1: Comparison of results of total no samples subjected for cytology for malignant cells (30), with Conventional Smear and cell block using chi Square Test

X2=4.80; p=0.028460 (significant)

| Technique        | Malignancy (+) | Malignancy (-) | Total |
|------------------|----------------|----------------|-------|
| Cell block       | 14             | 3              | 17    |
| Conventional Smears | 06             | 11             | 17    |

Table 2: Comparison of results of Fluid Samples positive for Malignant Cells (17) with Conventional Smear and Cell Block using Chi Square Test

X2=7.77; p=0.005308 (significant)
Table 3: Analysis of discrepancies between CB & CS METHOD for fluid cytology

| Sl. no. | Features    | CS METHOD No. (%) | CB METHOD No. (%) |
|---------|-------------|-------------------|-------------------|
| 1.      | Benign      | 62(86.11%)        | 58(80.55%)        |
| 2.      | Suspicious  | 04(5.55%)         | 0                 |
| 3.      | Malignant   | 06(8.4%)          | 14(19.45%)        |
| Total   |             | 72(100%)          | 72(100%)          |

Table 4: Table 2 comparison of results of total no of fluid samples subjected to conventional smear and cell block using chi square test

| Technique            | Malignancy (+) | Malignancy (-) |
|----------------------|----------------|----------------|
| Cell block           | 14             | 58             | 72             |
| Conventional Smear   | 06             | 66             | 72             |
|                      | 20             | 124            | 144            |

X²=3.72; p =0.053889(not significant)

**FIG. 1:** Photomicrograph of conventional smear of a case of Ca Ovary Positive for malignant cells.

**FIG. 2:** Photomicrograph of cell block of same case of Ca Ovary showing architectural arrangement and features of malignancy (better appreciated in comparison to conventional smear)
**FIG. 3:** Photomicrograph of conventional smear of benign smear with cellularity rich in lymphocytes (40x).

**FIG. 4:** Photo micrograph of cell block in a benign effusion showing many inflammatory cells and reactive mesothelial cells and haemorrhaghic material (40x).

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