Diagnostic efficacy of cell block method in correlation with conventional cytomorphology in pleural fluid samples

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A B S T R A C T

Introduction: Effusion cytology have gained acceptance worldwide in analysing body fluids, and a positive diagnosis rendered cytomorphology is often considered as a definitive diagnosis for further work up. However due to the known limitations, diagnostic problems arise in routine practice to differentiate reactive versus atypical mesothelial cells and malignant cells by the routine conventional smear (CS) method, thereby warranting adjunct studies like cell block techniques to enhance its efficacy.

Aims: To compare and analyse the cytomorphological features of the conventional cytosmears with those of the cell block sections and also to assess the utility and sensitivity of the cell block method in picking up the definitive diagnosis of pleural effusions.

Materials and Methods: The study was conducted in the cytology section of the Department of Pathology, MGCRI, SBV, Puducherry. Overall 50 pleural fluid samples were subjected to diagnostic evaluation for over a period of 18 months. Along with the conventional smears, cell blocks were prepared from the remnant fluid using Thromboplastin- pooled plasma method. Histopathology samples were obtained in feasible cases.

Results: Cellularity yield was low in cytosmears and was enhanced when combined with cell block method especially in non diagnostic /hemorrhagic samples, suspicious cases for malignancy, the efficacy was increased by a margin of 20 % more by the cell block method. Also pattern analysis was possible in Cell block and to categorize primary versus metastatic.

Conclusions: The cell block method provides high cellular yield, architectural patterns analysis, morphological features and aids in categorizing primary and metastatic effusions thereby, increases the efficacy of effusion cytology serving as a bridge between cytology and histopathology.

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1. Introduction

Effusion cytology had gained universal acceptance in evaluating body fluids especially of exudative nature rather than transudate. However the routine conventional cytology of body fluids has varied efficacy in terms of sensitivity and specificity due to known limitations. Since majority of serous cavity effusions has shedded lining mesothelial cells in the effusion, categorizing it into malignant or reactive mesothelial cells is a diagnostic challenge to Cytopathologist on conventional cytological smears. The major reason being many similar mimickers often warranting clinic-radio-pathological correlation. As the serous cavities are lined by mesothelial cells, its reactions to Research studies suggested that cell block (CB) technique when combined with Conventional smears enhances the efficacy of effusion cytology. Though CB method is being followed centuries back, it had gained less popularity due to various logistical reasons where technical expertise is one among the major reasons. In Literature there are around 16 different methods to prepare cell block each method having its own pros and cons. In recent past, Thromboplastin-pooled plasma procedure of preparing CB had become common which proved to be significantly

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efficient in effusion cytology of pleural fluid.

Research studies had been carried out in comparing CB prepared from Thromboplastin method with routine conventional cyto smears in all body fluids and proved to enhance the efficacy of pulmonary fluid cytology by a margin of 10-15%. With this Literature backup, the present study was carried with a novel aim in the Department of Pathology, Mahatma Gandhi Medical College & Research Institute, Puducherry, where the pulmonary fluid was subjected to study and the CB features were compared with routine cyto smears and we observed interesting observations.

2. Materials and Methods

The study was conducted in the Department of Pathology, Mahatma Gandhi Medical College & Research Institute, Sri BalajiVidyapeeth (Deemed to be University), Puducherry. The study was conducted for a period of 16 months from September 2018 to November 2019. Pulmonary fluid samples was received in the cytology laboratory from the clinical departments for routine cytopathological examinations. Among the received samples, fluidswith exudative nature was subjected to our study since Cell block technique is feasible only on exudative fluids in term of sediment button formations. Routine conventional smears was prepared in the laboratory using Pap stain, Hematoxylin and Eosin stain. Cytomorphology was analysed in terms of cellularity, nuclear features, pattern, background etc. Based on these features, thecyto smears were categorized as

1. Unsatisfactory smears for evaluation (due to haemorrhage obscuring, low cellularity, poor cell preservation)
2. Benign nature inclusive of pus of infective etiology /abscess (based on cytomorphology and background)
3. Suspicious or possibility of malignancy (based on 3D clusters, peripheral arrangement of nuclei, papillaroid clusters, etc)
4. Malignancy features

2.1. Cell Block method

The remaining remnant fluid which was to be discarded after the routine Conventional smear was subjected for our study for further cell block technique. Thromboplastin – pooled plasma method was followed in preparing Cell block as per the standard operating procedure of the Laboratory. The fluid sample was centrifuged with 3000rpm for 15 minutes and 3 to 4 drops of Thromboplastin reagent was added to the centrifuged sample and shaked gently. After a while 3 to 5 drops of pooledplasma which was kept in freeze was added and the tube was closed with a cotton plug and kept in erect position for overnight. Next day the supernatant fluid was discarded and the cell block button was subjected to paraffin block embedding and microtome section cutting.

Finally the cell block slide was stained with Hematoxylin and Eosin stain and evaluated.

2.2. Histopathology

Biopsy evaluation being the gold standard method of diagnosing pathological lesions, we received subsequent pulmonary biopsy in 25 cases. The observations were correlated with those of cell block diagnosis and conventional cyto smears

2.3. Statistical analysis

The prepared cell block slides was e valuated for its morphology and it was analyzed and correlated with the of routine conventional smear to calculate p value using Mmcemars test, keeping histopathology as gold standard (in available cases).

3. Results & Observations

The study was carried out for a period of one year and six months at Department of Pathology, Mahatma Gandhi medical college & Research Institute, SBV (Deemed to be University), Puducherry. Pleural fluid of exudate nature was included and almost Fifty pleural fluid samples were subjected to the study for routine conventional cyto smears and subsequent cell block method techniques. All the clinical parameters were documented in proforma format and analysed

3.1. Age

The age of the patients ranged as young as 18 years to as old as 90 years. However maximum number of samples were from the age group between 40 – 60 year age group (80%), the average age being 55 years of age. The least number of samples were obtained from the age group of 18 – 25 years (9%).

3.2. Gender

Pleural fluid from male patients (n=35) outnumbered the female patient samples(n=15). Majority of samples were of unilateral effusion except in 2 cases (both females) where bilateral effusion was encountered.

3.3. Morphological patterns (conventional smears vs cell block sections)

The conventional cytosmears were evaluated as per universal guidelines for Effusion cytology drafted by Academy of Cytopathologists
3.4. Cellularity

Cellularity was high in cell block sections when compared to cyto smears. In cytosmears 10 cases (20%) were reported as unsatisfactory or non-diagnostic for opinion due to factors like haemorrhage, obscuring blood, poor preservation of cells, etc. But among the 10 cases, cell block yielded good cellularity in 8 cases (80%) thereby a definite opinion could be given. Thus cell block proved to be highly effective in cases of unsatisfactory smears on conventional cytology as shown in Table 2.

3.5. Pattern analysis

Cyto smears predominantly showed clusters, monolayered sheets and papillary arrangements in few cases. However, all cases overlapping of cells. In Cell block sections architectural patterns including glandular, acinar, sheets, three-dimensional cell clusters, papillary clusters and cell balls with peripheral nuclear arrangements were appreciated in 27 cases including malignancy and suspicious category.

3.6. Benign vs malignant

Conventional cyto smears showed 10 cases as unsatisfactory which turned out to be benign in 4 cases and malignant in 5 cases in cell block study. 15 cases which showed benign features in cytosmears subsequent cell block showed only 10 cases as benign and rest 5 cases turned out to be malignant correlating with available biopsy samples. Thus the sensitivity and specificity of cytosmears in benign cases ranged only 65%.

Among 15 cases reported as malignancy and 20 cases with suspicion of malignancy in cytosmears, 23 cases correlated with subsequent cell block and thus cytosmears had a specificity of 70% in picking up malignancy lesions.

3.7. Primary versus secondary

Among the 25 malignant cases (n=50) reported, in 8 cases the effusion was due to metastasis and 2 cases were of mesothelioma. Rest 15 cases the primary site of origin was from lung, majority being adenocarcinoma of lungs. Among the 8 metastatic effusion cases, primary site could be identified only in 4(50%) cases and cell block picked up the primary site in all 8 cases thereby having high efficacy. In females, the primary was from breast carcinoma (3 cases), carcinoma stomach in 2 cases and pelvic malignancy in 3 cases.

3.8. Histopathology

Among the 50 cases analysed, subsequent histopathology specimens was available in 25 feasible cases with certain indication and all were of malignant pathology. In rest 25 cases, the clinician started treatment based on the cell block reports. The observations of cell block correlated well with histopathology thereby giving 100% efficacy as demonstrated in Table 2.

3.9. Statistical analysis

The results were compared keeping histopathology as gold standard (in available cases). With regard to benign and malignant lesions, cell block was statistically significant with a p value of 0.003 where cytosmears had a p value of 0.005 in case of malignant and 0.05 in case of benign lesion as shown in Table 3.

4. Discussion

Effusion cytology serves as an integral part in evaluation of body fluid from serous cavities. Effusion cytology includes conventional cytosmears, cytocentrifuge smears, cell block sections and cytospin methods. But due to limited resources either of the above method is being followed among Pathologists. Effusions may be pleural, ascitic, synovial, cerebrospinal fluid. Studies showed that the incidence of pleural effusion is high in Tropical regions due to various reasons and the efficacy of cytosmears in debatable often warranting invasive surgeries to arrive at diagnosis.

The major problem encountered in cytosmears in processing of fluids, blood obscuring, overlapping of cells etc. On cytomorphology, differentiating between reactive versus malignant cells is a great diagnostic challenge even to an experienced Cytopathologist due to many similar
Table 1: Age and gender distribution of received pleural fluid samples

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of cases</th>
<th>Males</th>
<th>No. of cases</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25 years</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>26-50 years</td>
<td>12</td>
<td>12</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>&gt;50 years</td>
<td>28</td>
<td>28</td>
<td>5</td>
<td></td>
</tr>
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</table>

Table 2: Analysis of CB vs CS with histopathology as baseline

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Benign (n=50)</th>
<th>Suspicious (n=50)</th>
<th>Malignancy (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional smears (n=50)</td>
<td>15</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Cell Block (n=50)</td>
<td>9</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>Histopathology (n=25)</td>
<td>-</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 3: Comparison CB vs CS in identifying Primary origin of pleural fluid using histopathology as baseline

<table>
<thead>
<tr>
<th>Procedure/Method</th>
<th>Mesothelioma</th>
<th>Breast ca</th>
<th>GIT</th>
<th>P-value (McNaemer test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell block</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0.003</td>
</tr>
<tr>
<td>Conventional smear</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Fig. 2: Cytosmears showing three dimensional clusters of suspicious cells in pleural fluid, Pap, 40x

Fig. 3: Cell block papillaroid clusters with atypia in pleural fluid, H & E, 40x

Fig. 4: Gross image of lung carcinoma showing grey white nodule with infiltration
Fig. 5: Histopathology showing acinar pattern bizarre shaped cells with pleomorphic nuclei and mucin- Adenocarcinoma lung, H & E, 40X Age and gender distribution of received pleural fluid samples

mimickers and both exhibit pleomorphism. It had been universally accepted that pleural effusion of any form in Pathognomic and needs to be evaluated thoroughly, since majority turn out to be malignant effusions.

Bahrenburg and his coworkers had formulated cell block method in early 1890s and thereafter it gradually gained popularity in various phased. Researchers have attempted to use cell block method as an adjunct to cytosmears in pleural fluid samples and proved to be useful. Though with more pros, cell block also has its own cons especially in losing material while processing. Lack of expertise being a major lacunae in carrying out cell block technique, Thromboplastin – pooled plasma method serves as simple, cost effective method with minimal hands on experience and less chance for tissue loss and often obviates the need for explorative surgery.

4.1. Advantages of cell block (Thromboplastin-pooled plasma method)

i) Recognition and analysis of patterns similar to histomorphology which cannot be done in cytosmears. ii) Scope to take multiple tissue sections as and when required for routine staining, special stains and provision immunohistochemistic procedures. iii) Minimal chance for obscuring hemorrhagic background. iv) Provision of storage slides for slide collection and for retrospective analysis whereas Storing of cytosmears is difficult as stain fades off rapidly.

In the present study majority of cases were males (35 cases) with male to female ratio of 3:1. Similar findings were also observed by Radhika et al and Nathan et al.\(^1,2\) The reason attributed to male preponderance is lifestyle, smoking and increased exposure to pulmonary toxic agents causing decline in immune status. With regard to females, the major cause being malignant effusion due to carcinoma breast which is again a common condition in this part of the country.

Conventional cytosmears showed unsatisfactory (Figure 1) in 10 cases (20%) with a sensitivity of 65% whereas subsequent cell block showed good cellularity (80%). Similar findings were observed by Price et al.\(^3\) The reason for this is stated that improper processing of fluid, irrational fixation and handling and lack of expertise.\(^4,5\) Thus it is evident that cell block proved to be highly efficient in picking up cellularity in non diagnostic samples.

In the present study, conventional smear showed overlapping clusters, nuclear pleomorphism with suspicion of malignancy in 25 cases (Figure 2). Subsequent cell block showed malignancy (Figure 3) which correlated well with histopathology findings rendering 100% specificity (Figure 4) similar findings were observed by Radhika et al and Zito et al.\(^1,6\)

Histopathology sections showed acinar, clear cells with mucin and nuclear pleomorphism in cases of Adenocarcinoma (Figure 5).

Apart from high efficacy, cell block also aided in categorizing malignant pleural effusions into primary and metastatic effusions. In the present study effusion due primary malignancy was higher than metastatic (from GIT > Breast > pelvic organs). Similar findings were observed by Yazie et al.\(^7\) The reason attributed being clonal expansion and reaction of mesothelial cells to the noxious stimuli.\(^2\)

5. Conclusion

The present study states that efficacy of conventional cytosmears in effusion cytology of pleural fluid in relatively low and when combined with cell block technique, the efficacy increases marginally by 15 to 18%. The study also emphasizes that the cellblock method, by using Thromboplastin - pooled plasma technique was a simple, reliable, cost effective method with minimal chance for losing material. Cell block also provides scope for marker studies and retrospective analysis, assessing staging and prognosis of the condition. We conclude by stating that cell block technique could be considered as a bridge between cytology and histopathology to arrive at definite diagnosis often obviating the need for exploratory invasive surgeries.

6. Source of funding
None.

7. Conflict of Interest
None.
References


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