Diagnostic Utility of Pleural Fluid Cell Block Analysis in Malignant Pleural Effusion and Its Comparison with the Conventional Pleural Fluid Cytology – An Evaluation of 63 Cases in a Tertiary Care Hospital

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Abstract

In patients with pleural effusion (PE), conventional cytology (CS) is one of the first and useful step for diagnostic evaluation. When there is suspicion of malignancy (Suspected Malignant Effusion), there are some limitations of conventional cytology. Cell block (CB) method was developed to overcome such situation. The CB is also very useful for final categorization of tumors by performing IHC on it. This has a great implication in final therapy.

The aim of this study is to establish the diagnostic utility of the cell block analysis in malignant pleural effusion (MPE) and to compare it with the conventional cytology study.

It was a descriptive observational study, conducted in Department of Pathology, CMSDH from June 2018 to May 2019. In all 63 cases with mean age of 54.36 a cross-sectional study was conducted. All the patients having pleural effusion with a suspicion of malignant etiology underwent thoracocentesis from June 2018 to May 2019. All samples are subjected to routine biochemical analysis, conventional cytology and cell block histology. The histological and cytological analysis was done.

36 patients who were finally diagnosed as having malignant pleural effusion were included in the study. Final diagnosis of malignancy were found as 27 adenocarcinoma, 5 malignant mesothelioma, 3 were metastatic breast cancer and 1 was metastatic papillary carcinoma. Among 36 malignant pleural effusion diagnostic yield of cell block & conventional cytology were 63% & 61.2% respectively. Combined cell block & conventional cytology improves the diagnostic yield to 71.2% (P < 0.001).

In malignant pleural effusion, cell block provides more diagnostics yield than conventional cytology while application of both techniques significantly increases the diagnostic yield.

Keywords: Cell block, malignant pleural effusion and conventional cytology.

INTRODUCTION

Though pleural effusion is one of the clinical signs of patients having malignancy, but its definitive diagnosis whether the fluid is negative for malignancy or positive for malignancy is very important. Accurate diagnosis of malignant pleural effusion is not only important in planning the appropriate management but also prognostication of the malignancy [1-3]. In patients having pleural effusion thoracocentesis is done and the fluid is submitted for cytological study. Cytological study is
extremely useful in suspected malignant pleural effusion as it provides a diagnostic rate of 60% ranging from 40% to 87% [1-4]. The challenges of obtaining an accurate diagnosis in conventional cytology (CS) are indistinct cell morphology, overlapping of cell, abundance of inflammatory cells, paucity of representative cells, smearing artifacts and cell loss [5]. To overcome these limitations cell block was developed to provide better tissue architecture and morphological features that help in differentiating between malignant and non-malignant cells. The other advantage of cell block is that it can be further utilized for special stain & immunohistochemistry [6], further enhancing the definitive diagnosis of malignancy. In this study diagnostic utility of CB in MPE and its advantages over CS (Conventional cytology) is studied.

Materials and method: The present study was a retrospective analysis of 123 patients with pleural effusion during the study period of 1 year (June 2018 to May 2019) in our institute.

Inclusion Criteria: Out of 123 patients, 36 patients having malignant effusion are included in the study.

Exclusion Criteria: The pleural fluids which are negative for malignancy are excluded. Written informed consent was obtained from all patients/patient relatives before thoracocentesis for diagnostic purposes. The study protocol was approved by our Institutional Ethics Committee.

For conventional cytology, 1.5 ml. of fresh pleural fluid was centrifuged at 2,500 rpm for 20 min and the supernatant removed. From the sediment direct smears are prepared and subjected for Leishman – Giemsa and papanicolaou staining.

For cell block, 15 ml. of fresh PF was centrifuged at 6000 rpm for 5 mins and then supernatant removed. Agar solution was added to the sediment followed by refrigeration to form a solid clot. The clot was fixed with 10% neutral buffer formation. Alternatively pleural fluid with high blood content (haemorrhagic fluid) thromboplastin plasma is used. Then a paraffin embedded block prepared. Histological slide cut and Hematoxylin & Eosin (H&E) stain was performed.

Results: We have evaluated 123 cases out of which 36 patients were diagnosed as malignant pleural effusion. In conventional cytology out of 36 cases 24 cases have the definitive cytological features of malignancy. This finding is further enhanced and confirmed in the CB slides. In 12 cases there is suspicion of malignancy in the conventional smear. In these 12 cases cell block plays a very important role for confirmation of the diagnosis of malignancy as it provides better tissue architecture and morphological features of the malignant cells. When the cell block study was taken in the account alone in 32 cases there is definitive diagnosis of malignancy was established and in 4 cases there is insufficient tissue material in the histology slides for definitive diagnosis. In these 4 cases diagnosis of malignancy was established by the definitive cytological features of malignancy in CS. Among the 36 cases male cases (26) were predominant with a male-female ratio of the age range was from 27 years to 63 years with an average of 45 years (Table 1) presenting symptom of the patients in the study were as following.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Number of cases (n=36)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest pain</td>
<td>30</td>
<td>83%</td>
</tr>
<tr>
<td>Cough</td>
<td>34</td>
<td>94%</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>18</td>
<td>50%</td>
</tr>
<tr>
<td>Weight loss</td>
<td>32</td>
<td>88%</td>
</tr>
<tr>
<td>Fever</td>
<td>12</td>
<td>33%</td>
</tr>
</tbody>
</table>

Table 1:- Presenting symptoms of patients in this study.

Table No 2:- Age group and gender comparison.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male (%)</th>
<th>Female (%)</th>
<th>M/F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (36)</td>
<td>26(72%)</td>
<td>10(27%)</td>
<td>2.6 %</td>
</tr>
</tbody>
</table>
The most common malignancy diagnosed was adenocarcinoma(27), 5 malignant mesothelioma, 3 metastatic breast carcinoma and 1 metaplastic papillary carcinoma of thyroid.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Total Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;=50 years</td>
<td>08 (30.7%)</td>
<td>04 (40.1%)</td>
</tr>
<tr>
<td>&gt;=50 years</td>
<td>18 (69%)</td>
<td>06 (60%)</td>
</tr>
</tbody>
</table>

Table-3: Distribution of diagnosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Total Case (n=36)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asenocarcinoma (n=27)</td>
<td></td>
<td>75%</td>
</tr>
<tr>
<td>Malignant Mesothelioma (n=5)</td>
<td></td>
<td>13%</td>
</tr>
<tr>
<td>Metastatic Breast Carcinoma</td>
<td></td>
<td>8%</td>
</tr>
<tr>
<td>Metastatic Papillary Carcinoma (n=1)</td>
<td></td>
<td>2%</td>
</tr>
</tbody>
</table>

Discussion: Pleural disease can be diagnosed by strict examination of the pleura itself (followed by biopsy) or by indirect evaluation of pleural fluid that accumulates in the pleural cavity. Thoracocentesis to retrieve pleural fluid is a very simple procedure and available in most of the hospitals. It is well accepted as an initial investigation. In malignant pleural effusion, cytological study from pleural fluid provides a diagnostic rate of 60%, ranging from 40% to 87% [1-4]. To enhance the diagnosis from cytology of pleural effusion, the sediment from centrifuges pleural effusion can be processed as cell block for histology.

Cell block technique has been described for nearly a century and is well-known among cytopathologists, it is still under prescribed by clinicians, until recently where there is increasing demand for cell block analysis by clinicians. We conducted this study to explore the benefits of cell block study in malignant pleural effusion.

Before embedding into paraffin blocks the sediment cells are mixed with fixatives, which help in birding of these sediment cells. There are many fixative substances used like formalin, alcohol-formalin, alcohol-acetic-acid formalin, agar, plasma thrombin clot, cytolyte-prefixed thrombin clot, Histo-Gel TM and inverted filter sedimentation [5-8, 13]. Some studies have evaluated cell morphology and IHC performance of cell block using different fixatives-[6,13], there is no consensus guideline in this process, leaving the choice up to each institute based on availability and cost-affordability. In our study most of the cases formalin is used to form a solid clot along with thromboplastin plasma (Plasma thrombin methods) in case the effusion fluid is haemorrhagic or having high blood content. The cost for cell block preparation was low. In malignant pleural effusion diagnosis, cell block has certain advantages over conventional cytology. In conventional cell cytology in proper smear, fixation and staining techniques can cause cell overlapping or overcrowding, cell loss, artifacts and poor background staining white these are less frequent in cell block [5-8]. Compares with conventional cytology cellularity is higher and is concentrated in one small area that can be evaluated at a glance with all cells lying in the same focal plane of the microscope [9, 10]. In addition cell block provides better cellular morphological details, such as better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin, there is also less difficulty in microscopic observation, in spite of the presence of excess blood in the background [10]. Adenocarcinoma cells especially from the lung, breast as gastrointestinal tract, may not clearly exhibit cellular morphology of malignant, better morphological details and tissue architecture pattern are required for diagnosis [5,9, 10]. Conventional fluid cytology has the limitation of a lack of tissue architecture. In conventional cell study, predominantly singly scattered cells are found ,where as architecture patterns such as glands, sheets, three dimensional cell clusters and cell balls are commonly demonstrates in cell block resulting in increased sensitivity of diagnosis of malignant pleural effusion.
by cell block method [10]. One of the major challenges in conventional cell cytology is discrimination of reactive mesothelial cells and malignant cells as the former may express large irregular nucleoli, coarse chromatin and enlarged nuclei mimicking malignancy [5, 7, 8, 9, 10]. In contrast, in cell block the nucleoli are not as prominent as in conventional fluid cytology and the pseudo-acinar or acinar structures can be better appreciates [9, 10]. Another advantage of cell block is it can be stones and multiple sections can be taken for routine staining, special staining, IHC staining and also molecular testing [14,15]. In contrast storage of conventional cytology is a practical problem. Despite of so many advantages cell block has a risk of losing material during preparation especially in the case of fixation technique [16] that might explain negative cell block results but positive cell cytology result in some cases. Similar to our results, previous studies showed an additional diagnostic rate of cell block to cell cytology around 10-15% in malignant pleural effusion. [5, 7, 8, 10, 17].

Conclusion: To conclude in our study cell block provides a higher diagnostic performance to cell cytology in malignant pleural effusion. While if both techniques cell block & conventional cell cytology are used, it can significantly increase the diagnostic yield than the cell block study alone.

References
14. Shivakumarswamy U, Arakeri SU, Karigowdar MH, Yelikar B. Diagnostic utility of the cell block method versus the


Figures:

Figure 1: Photomicrograph of pleural fluid cytology of adenocarcinoma of lung. (PAP stain, High power view)
Figure 2: Photomicrograph of cell block of adeno carcinoma (H &E stain, Low power view)