A Cytological Study to Differentiate Between Reactive Mesothelial Cells and Malignant Cells in Effusions

T. Bharath1 T. Satya Prakash Venkatachalam2
1Assistant professor, department of pathology, Nimra institute of medical sciences, AP, India, 2Professor & HOD, Department of Pathology, GSL medical college, AP, India.

Abstract: The excessive accumulation of fluid in the body cavities is called as effusion. Since the effusion fluids can be the face of the underlying pathology mostly due to Infections and malignancies the cytological evaluation of these serous effusions is performed mainly to establish the presence or absence of malignancy. Cytological differentiation of reactive mesothelial cells from malignant cells is often difficult by conventional cytology and hence Immunocytochemistry (IHC) is more sensitive and specific than other methods including the ease of availability. The aim of the present study is to analyze the samples of body fluids with a stress on differentiating between reactive mesothelial cells and the malignant cells.

Materials & methods: Fluid samples that were received at the hospital cytology section of GSL General Hospital during the period from October 2013 to September 2015. Cytological smears and cell blocks were prepared and evaluated by cytomorphology in all the cases, cytochemistry and immunohistochemistry (IHC) was done to identify cells as per the merit of the case using at least two IHC markers. It was proposed to study a minimum of 100 samples

Results: A total number of 335 samples of effusions were received out of which, 235 were opined as inflammatory effusions, 40 cases were opined as malignant effusion and 35 cases showed reactive mesothelial cells. A peer study was conducted by 3 different observers and the suspicious cases were subjected to IHC with Calretinin and EMA so as to determine the nature of these suspicious cells whether they are of mesothelial lineage or of epithelial in nature.

Conclusion: This study has given an insight as to the morphological variations that a reactive mesothelial cell can show nearly bordering on to malignancy and that the fact has to be kept in mind while reporting effusion cytology. Calretinin unfortunately gave negative staining in all the cases that were opined as reactive mesothelial cells in spite of the reagent gave an excellent staining in control. The reason for this uniform negativity is unfathomable and requires further evaluation. Where as EMA yielded reliable results.

Key words: Effusions, Mesothelial cell, Calretinin, EMA

I. Introduction

Internal visceral organs of all vertebrates are located in cavities, collectively called as “Body cavities” and the thin layer that lines these cavities is called as “Mesothelium”. Hence these cavities are also expressed as mesothelial cavities. In physiological state there is minimal amount of serous fluid present in these cavities. (1)

However, in pathological states fluid accumulates in these cavities in significant quantities is called as effusion and analysis of this effusion fluid may give valuable information so as to arrive at a diagnosis. Conventionally, fluids obtained from body cavities are subjected to pathological, microbiological and biochemical analysis. Infections and malignancies, both primary and metastatic, are the most common causes for these effusions and hence cytological evaluation of serous effusions is performed mainly to establish the presence or absence of malignancy.

Cytology is an important alternative study to histology as it is the first best chance in the interpretation and diagnosis of malignancy. Body fluids constitute an ideal tissue culture medium where in desquamated cells, both benign and malignant, may proliferate freely and stay viable even after shedding. The mesothelial cells are so dynamic that they take many forms and sometimes so bizarre, that it becomes very difficult to categorically identify these reactive mesothelial cells with certainty. Many a times they mimic malignant cells and cast difficulty. (2)

The history of serous effusion cytology can be traced back to the 19th century. (3) Peritoneal washing cytology (PWC) was introduced in the 1950s as a way to identify microscopic spread of cancer not visible by gross inspection of the peritoneal surface. (4)

The following steps may help in increasing diagnostic accuracy: (5)
I. To obtain the appropriate clinical data before coming to any conclusion is of crucial importance.
II. It is practical to scan the slide at low magnification first, ideally without knowledge of the clinical history, thereby forming a general impression of the pathologic process present.
III. Avoid the common mistake of concentrating too much on individual cell atypia and morphology, ignoring the patient’s history and overall cellular background that often results in false positive diagnosis.
IV. The temptation to immediately study the cells at high magnification is another fallacy, which should be avoided in all cases.

Special stains like Periodic acid Schiff (PAS), Mucicarmine and Alcin blue stains are helpful in the field of diagnostic cytology, but lack specificity and sensitivity. Ancillary techniques such as electron microscopy, flow-cytometry and morphometry have been used as adjuncts to solve the ambiguity in cytological differentiation between reactive mesothelial cells and malignant cells. But they are also not free from limitations, thus Immunocytochemistry (IHC) is more sensitive and specific than other methods including the ease of availability.

A posse of markers has been studied and is now available not only to help in the identification of malignant cell deposits in effusions but also to differentiate them from mesothelial cells. The present study has been undertaken to analyze the samples of body fluids with a stress on differentiating between reactive mesothelial cells and the malignant cells.

II. Aims and objectives
1) To analyze the samples of effusions received during the period from October 2013 to September 2015 at GSL Medical College, Rajahmundry as per the existing standard procedures.
2) It is proposed to study not less than 100 samples during the period of study.
3) To establish a method in differentiating reactive mesothelial cells from malignant cells in doubtful cases by using histochemical / cytochemical methods and immunohistochemical / immunocytochemical markers that help in arriving at an accurate diagnosis.

III. Materials and methods

Fluid samples that were received at the hospital laboratory cytology section of GSL General Hospital during the period from October 2013 to September 2015. They were properly labeled for identification. Relevant information with regards to clinical, biochemical, bacteriological and imaging studies was collected. These samples were analyzed as per the standard cytological procedures as describes in Koss Diagnostic Cytology and its Histopathologic Bases. (1) Cytological smears and cell blocks were prepared after centrifugation of collected fluids. These were evaluated by cytomorphology in all the cases cytochemistry and immunohistochemistry (IHC) was done to identify cells as per the merit of the case using at least two markers to eliminate error in sensitivity.

3.1 Sampling techniques employed

Paracentesis (Collecting serous fluids)
Although a serous effusion may be removed at the time of surgical exploration it is usually sampled by the relatively simple procedure of inserting a wide bore needle (under local anesthesia) through the body wall into the fluid containing cavity (1). The fluid was collected into a clean dry sterile container and sent to the laboratory as soon as possible. When the fluid could not be sent immediately, it was preserved in a refrigerator at 4°C and was not allowed to freeze. No anticoagulant or fixative was added to the fluid. (1)

Cytological examination was carried out on centrifuge deposit smears stained by Leishman’s stain and H& E/ Pap stains. The remaining unstained air dried smear was kept aside for staining by special stain techniques like Periodic acid Schiff stain (PAS), if warranted. Cell blocks were also made simultaneously from the centrifuge deposits and thin sections were cut from the paraffin blocks made, stained by H and E method and studied.

IV. Results

A total number of 335 samples of effusions were received during the period of study conducted from October 2013 to September 2015. Out of these effusions 203 were pleural fluids, 125 were ascitic fluids and 7 were pericardial fluids. In this study the age ranges from 13 to 90 with a peak incidence between the age group of 50 to 59 years.
Gross examination of the samples revealed that 10 samples (3%) were found to be hemorrhagic and remaining 324 samples (97%) were found to be of non-hemorrhagic.

Out of the 335 samples, 235 were opined as inflammatory effusions in view of presence of only inflammatory cells, 40 cases were opined as malignant effusion in view of the presence of malignant cells with unequivocal morphology, while as the remaining 35 cases showed reactive mesothelial cells.

To bring in more authenticity and reliability a peer study was conducted by 3 different observers who have more than seven years of experience in the field of diagnostic pathology.

Since only 26 samples showed good cell yield in cell block preparations, the archival smears pertaining to these 26 cases were retrieved and were subjected to peer study.

The study revealed that there was concordance between all the 3 observers in 4 cases for malignancy and 6 cases for reactive mesothelial cells and discordance was observed in 16 cases.

These cases were categorized as suspicious cell morphology and were subjected to IHC marker study to determine the nature of these cells, whether they are of mesothelial lineage or of epithelial in nature.

Calretinin and Epithelial membrane antigen (EMA) were chosen as IHC markers for mesothelial cells and malignant epithelial cells respectively, for this study.

Calretinin is an antigen expressed by both benign and malignant mesothelial cells to a tune of 95% - 100% of cases and Epithelial Membrane Antigen (EMA) is a marker for epithelial neoplasm with an incidence rate of 100% in the study done by Murghan.

Out of the 16 cases 8 cases showed negative staining for Calretinin but were found to be positive for EMA. Hence these cases have been confirmed to be adenocarcinoma deposits.

Two cases were found to be negative for EMA and hence these two cases were considered to be negative for malignant cells.

![Figure 1,2: Normal mesothelial cells(40X H&E)](40XH&E)  ![Figure 3: Reactive mesothelial cells(40XH&E)](40XH&E)
In this present study pleural fluids were found to be more in number when compared to ascitic and pericardial fluids. The pattern of effusions seen in this present study is in consonance with studies by Su XY, Shield PW who also reported that the majority were pleural fluids with an incidence of 70.8% and 83.7% respectively.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>PLEURAL FLUID</th>
<th>ASCITIC FLUID</th>
<th>PERICARDIAL FLUID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>60.6%</td>
<td>37.3%</td>
<td>2.1%</td>
</tr>
<tr>
<td>Su XY</td>
<td>70.8%</td>
<td>26.5%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Shield PW</td>
<td>83.7%</td>
<td>16.3%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Out of these 335 samples, 5 samples (1.5%) were found to be transudates showing very scanty cellularity. Microscopic examination of the smears though was not met with any difficulty while studying inflammatory effusions and effusions where the mesothelial cells showed classical textbook like appearance, reactive mesothelial cells posed a problem in differentiating them from malignant cells.

In this study the reactive mesothelial cells showed features near to neoplasm like high N:C ratio nuclear pleomorphism, Multinucleation and bizarre forms. This problem has been encountered by many workers also and has lead to search for alternative methods for differentiating reactive mesothelial cells from malignant cells. Though initially special histochemical stains came to the stead but with limited utility, immunohistochemistry has taken over the charge of arriving at a diagnosis with certainty.

The same results were encountered in this centre also. The peer study conducted in connection with this present study also resulted in discordance between three investigators. Diligent search to find whether any such

---

**Figure 4:** Suspicious cell clusters (40X H&E) **Figure 5:** Malignant cells in effusions forming clusters and acini/glandular patterns (40X H&E)

**Figure 6:** Cell membrane positivity of malignant Cells (EMA) 40X

**Figure 7:** Calretinin negativity for reactive mesothelial cells 40X

---

V. Discussion

In this present study pleural fluids were found to be more in number when compared to ascitic and pericardial fluids. The pattern of effusions seen in this present study is in consonance with studies by Su XY, Shield PW who also reported that the majority were pleural fluids with an incidence of 70.8% and 83.7% respectively.
peer studies were conducted previously was surprisingly met with a naught and hence this peer study appears to be the first of its kind!

In this study EMA was found to be of great use in confirming the diagnosis of adenocarcinoma deposits.

However in this study Calretinin was found to be uniformly negative in cell block preparations irrespective of whether they are malignant effusions or benign ones. Since the control showed adequate and good result, it is difficult to explain the negativity in cell block sections. Since many studies showed Calretinin positivity to be ranging from the highest of 100% to a lowest of 27% total absence of calretinin reactivity in this study cannot be construed to fall in the negative staining seen in 73% of case of the study which showed lowest positivity of 27%. This does not follow the rule of probability and stand to reasoning.

Neither there could be any procedural error in cell block preparation as many of these cell blocks showed positivity for EMA. Hence preparation of cell block cannot be blamed for the uniformly negative result for Calretinin that were opined as reactive mesothelial cells in spite of the reagent gave an excellent staining in control. The reason for this uniform negativity is unfathomable and requires further evaluation.

The negative results though are statistically incorrect but they may have to be taken in good stead by their face value and considered authentic.

VI. Conclusion

This study has given an insight as to the morphological variations that a reactive mesothelial cell can show nearly bordering on to malignancy and that the fact has to be kept in mind while reporting effusion cytology. Cross consultation is of great help in case of doubt. Calretinin alone cannot be relied upon as a marker for mesothelial cells and hence at least two markers for mesothelial cells should be included for identification of these cells.

EMA has been found to be a reliable marker for epithelial cells. However a second marker for epithelial cells has to be kept in reserve in cases high suspicion in spite of EMA negativity.

References