Section: Pathology

Original Research Article

IMMUNOHISTOCHEMICAL DIFFERENTIATION BETWEEN MALIGNANT CELLS AND MESOTHELIAL CELLS IN PLEURAL EFFUSION

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ABSTRACT

Background: Differentiating malignant from reactive mesothelial cells is challenging, particularly in pleural cytology, due to overlapping cytologic features . This study evaluates the effectiveness of EMA (Epithelial membrane antigen), Desmin and Calretinin in distinguishing mesothelial cells from malignant cells in cytologic effusions and provides a comparative analysis of their diagnostic performance.

Material and Methods: This study, conducted at Government Medical College, Thrissur, from January 2017 to June 2018, focused on pleural fluid samples from patients diagnosed with reactive mesothelial cells, atypical mesothelial cell proliferation and metastatic carcinoma.Samples were examined using both cell block and conventional smear methods. Immunohistochemistry (IHC) was performed on cell block sections with markers EMA, Desmin and Calretinin using the immunoenzymatic soluble complex method.

Results: The study included 56 patients, with a mean age of 62.9 years, and found that hemorrhagic effusions were most commonly caused by malignancy. The cell block (CB) method demonstrated significantly higher diagnostic efficiency for malignancy (75.5%) compared to conventional smear (46.9%), and combining both methods improved diagnostic accuracy. Immunohistochemistry (IHC) showed strong EMA expression in adenocarcinoma cases with a sensitivity and specificity of 100% ~~.and~~ Desmin positivity was seen in a subset of reactive effusions, with sensitivity and specificity being 36.8% and 89.1% respectively.

Conclusion: This study evaluates the use of EMA, Desmin and Calretinin to differentiate mesothelial cells from malignant cells in pleural effusions. It finds that combining smear and cell block methods improves diagnostic yield, with cell block preparation offering better cellular details. EMA shows 100% sensitivity and specificity in identifying malignant cases, while Desmin helps identify reactive mesothelial cells. The combination of EMA and Desmin enhances diagnostic accuracy, making them a reliable panel for challenging effusion cases.

Key Words: Immunohistochemistry, Pleural effusion, Mesothelial cells, Malignant cells, Epithelial membrane antigen, Desmin.

INTRODUCTION

Differentiating malignant from reactive mesothelial cells is challenging, especially in pleural cytology, due to overlapping cytologic features. While various antibodies aid this distinction, clinical research continues to seek a biomarker with high sensitivity and specificity.[1] Immunocytochemical and molecular methods on cell blocks or smears improve diagnostic accuracy.[2]Various studies have

demonstrated the utility of immunohistochemical markers, such as EMA (epithelial membrane antigen), Desmin, Calretinin, Fibronectin, CEA (carcinoembryonic antigen), Vimentin, P53, and Ki-67, in distinguishing reactive mesothelial cells from malignant cells. Among these markers, Calretinin, Desmin, and EMA have been identified as particularly promising for differentiating mesothelial cells from malignant cells in effusion cytology.[4-6] However, some mesotheliomas, like sarcomatous types, are rarely detected through effusion cytology. Diagnosing mesothelioma, particularly epithelioid types, requires panels of immunohistochemical markers with both positive and negative predictive value to ensure 100% sensitivity and specificity.[3]

This study assesses the effectiveness of EMA, Desmin and Calretinin in differentiating mesothelial cells from malignant cells in cytologic effusions and provides a comparative analysis of their diagnostic performance.

MATERIALS AND METHODS

This study was conducted in the Cytology Lab of the Department of Pathology at Government Medical College, Thrissur, a tertiary care institution, from January 1, 2017, to June 30, 2018, covering a period of 18 months. Pleural fluid samples from patients diagnosed with reactive mesothelial cells, atypical mesothelial cell proliferation and metastatic carcinoma were included in the study. Cell-poor effusions and cell block preparations, fluid samples negative for malignancy, grossly purulent fluid samples, and repeat samples were excluded. The study was approved by the Institutional Ethics Committee at Govt. Medical College, Thrissur. All eligible pleural effusion samples received were subjected to both cell block and conventional smear examinations. Fluid samples were collected in clean test tubes or containers. Half of each sample was allocated for the conventional smear method, while the other half was allocated for the cell block method. Conventional Smear Technique: Fluid samples were centrifuged at 3000 rpm for 10 minutes. A minimum of 3 smears were prepared from the sediment. Two smears were fixed immediately in 85% isopropyl alcohol and stained with Papanicolaou stain. The third smear was air-dried and stained with either Leishman or Giemsa stain. Cell Block Technique: The fluid was centrifuged at 3000 rpm for 10 minutes after adding fixative (Acid-Alcohol Formalin). The supernatant was discarded, and the sediment was treated with Acid-Alcohol Formalin overnight. The sediment was processed along with routine histopathological specimens. Paraffin-embedded sections of 4-6 µm thickness were prepared and stained with H&E stain. Microscopic examination was performed to categorize the slides as either benign or malignant based on cytomorphological features. Immunohistochemistry: Immunohistochemistry (IHC) was performed on cell block sections using the markers EMA, Desmin, and Calretinin, employing the immunoenzymatic soluble complex method. 4 µm thick sections were made on poly-L-lysine-coated slides. Slides were incubated at 37°C overnight and at 60°C for 1 hour, followed by dewaxing in xylene. Antigen retrieval was performed, and staining was carried out. Immunoreactivity was scored semi-quantitatively based on the percentage of cells stained and the intensity of staining. Data were entered into Excel sheets, and the specificity and sensitivity of each immunohistochemical marker were calculated using SPSS 16 statistical software.

RESULTS

A total of 56 patients who met the eligibility criteria were included in the study. The age of the patients ranged from 29 to 91 years. The most frequent age group in this study was 61–80 years (51.8%), with a mean age of 62.9 years. Out of the 56 cases studied, 17 cases (30%) were males, and 39 cases (70%) were females, with a male-to-female ratio of 1:2.1.[Table 1]

Effusions were categorized as hemorrhagic in 32 samples and non-hemorrhagic in 24 samples. The most common cause of hemorrhagic effusions was malignancy, accounting for 28 (87.5%) of all hemorrhagic fluid samples, while only 4 (12.5%) were reactive effusions. Out of the 49 malignant effusions, 28 (57.2%) were hemorrhagic, and 21 (42.8%) were non-hemorrhagic.

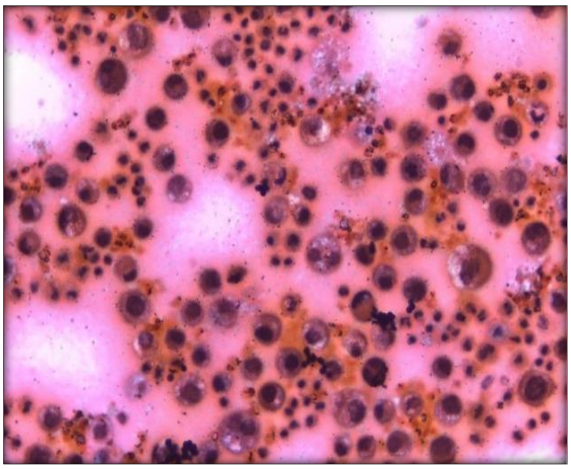
Cellularity is significantly better in cell block (CB) compared to conventional smear (CS), and nearly equal cellularity was observed in approximately 24 cases. Cytoplasmic features were superior in CB, nuclear features were enhanced in CB and architectural patterns were better appreciated in CB.

The efficiency of the cell block method in diagnosing malignancy was 37/49 (75.5%), compared to 23/49 (46.9%) for the conventional smear method. This difference was statistically significant (p-value -0.018). When combining the two tests, the diagnostic efficiency improved to 38/49 (77.6%), demonstrating a statistically significant increase in diagnostic accuracy compared to conventional smear analysis (p-value - 0.001).

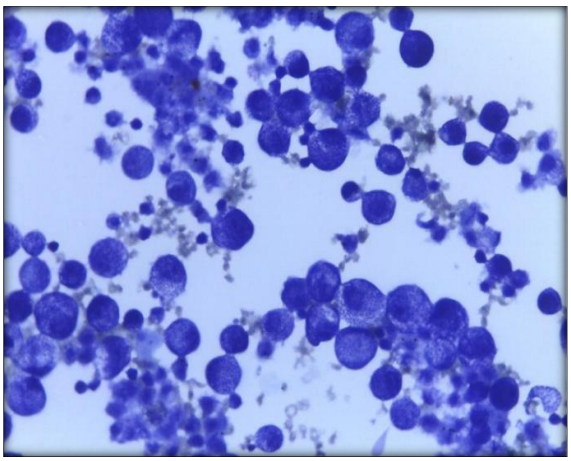
In Conventional Smear Diagnosis, 23 cases (41.1%) were adenocarcinoma, and 33 cases (58.9%) were reactive mesothelial hyperplasia.In Cell Block Diagnosis, 37 cases (66.1%) were adenocarcinoma, while 19 cases (33.9%) were reactive mesothelial hyperplasia. Immunohistochemistry (IHC) identified 49 (87.5%) adenocarcinoma cases and 7 (12.5%) reactive mesothelial hyperplasia.

When compared to conventional smear, cell block methods have demonstrated a sensitivity of 59.4%, specificity of 94.7%, positive predictive value of 95.6%, and negative predictive value of 54.5%.

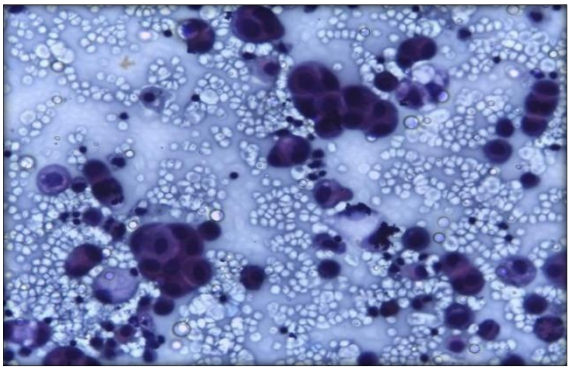
When both conventional smear and cell block methods are combined and compared with IHC diagnosis, the sensitivity increases to 77.5%, specificity reaches 100%, positive predictive value is 100%, and the negative predictive value is 38.8%. In 49 cases, strong and diffuse cytoplasmic expression of EMA was observed, with 70% of these cases showing an IHC score of 6. The sensitivity and specificity of EMA were both 100%, with a positive predictive value of 75.5% and a negative predictive value of 58.3%. For desmin expression in effusions, it was positive in 11 cases, of which 7 cases were exclusively desmin-positive. Among these, 42.9% (3/7) had an IHC score of 5, while 57.1% (4/7) had an IHC score of 6. The remaining 4 cases with IHC grade 4 exhibited strong and diffuse EMA positivity, indicating adenocarcinoma. The sensitivity and specificity of desmin were 36.8% and 89.1%, respectively, with a positive predictive value of 63.6% and a negative predictive value of 73.3%.



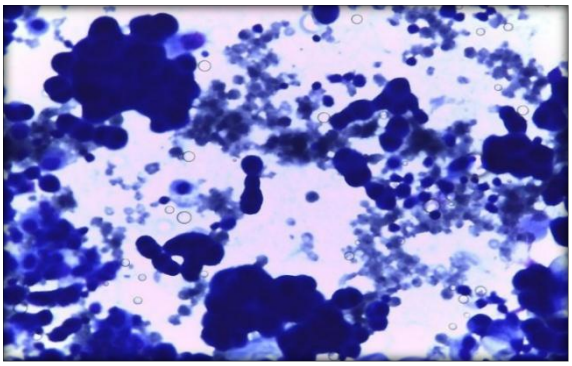
Conventional smear: pleural fluid sample showing mesothelial cells scattered singly in a bloody background (Papstain)



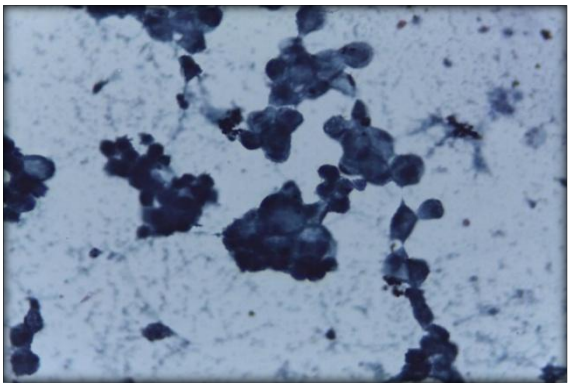
Conventional smear: pleural fluid sample showing mesothelial cells scattered singly in a bloody background (Giemsa stain)



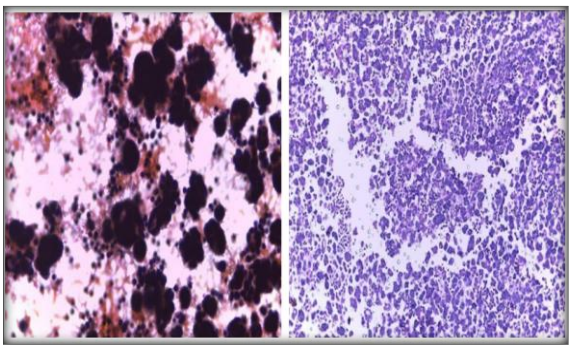
Conventional Smear: Pleural fluid showing Reactive mesothelial cells with cell window and cytoplasmic vacuolation (Pap Stain)



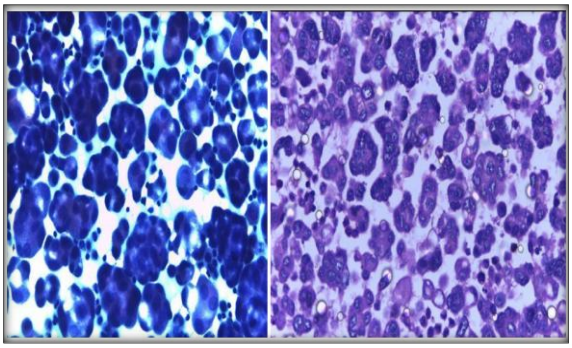
Conventional smeaer: pleural fluid showing reactive mesothelial cells with cell window (Giemsa stain)



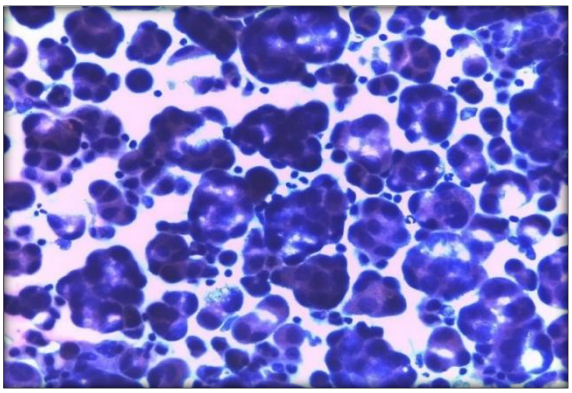
Conventional smear: Pleural fluid showing adenocarcinoma cells with a few signet ring cells (Giemsa stain)



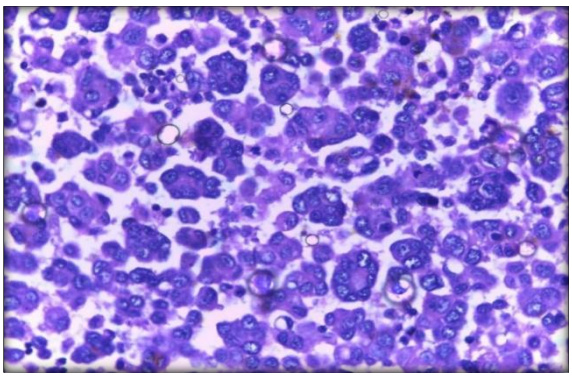
Cellularity in conventional smear compared to cell block



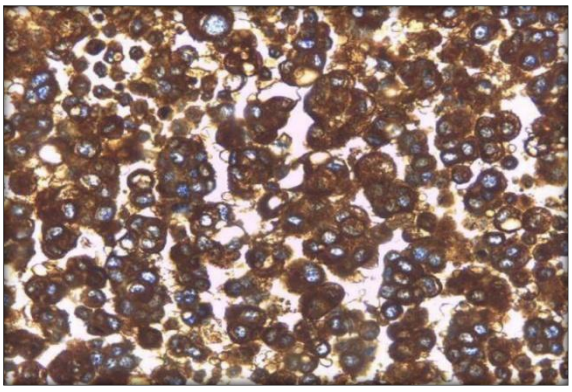
Pattern, cytoplasmic and nuclear features in conventional smear compared to cell block



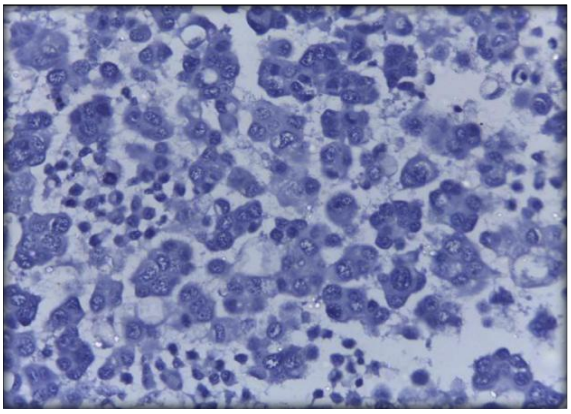
Adenocarcinoma in conventional smear (Papstain)



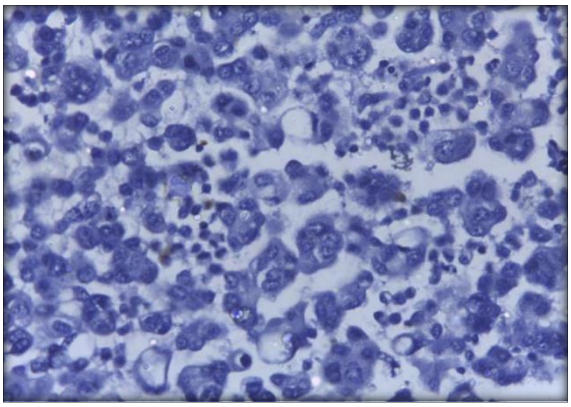
Adeno carcinoma in cell block (H&E stain)



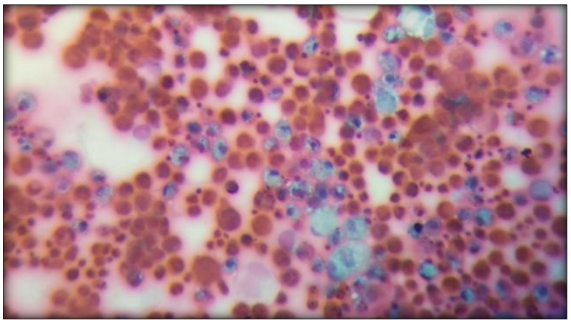
EMA Positivity in adenocarcinoma cells - diffuse strong cytoplasmic positivity. Staining Score:3; Intensity Score: 3. IHCS core: 6



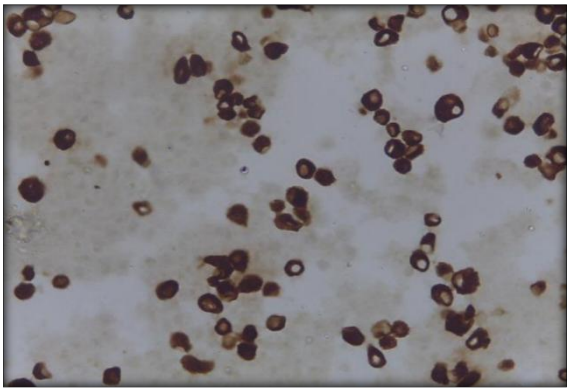
Desmin negative adenocarcinoma cells.



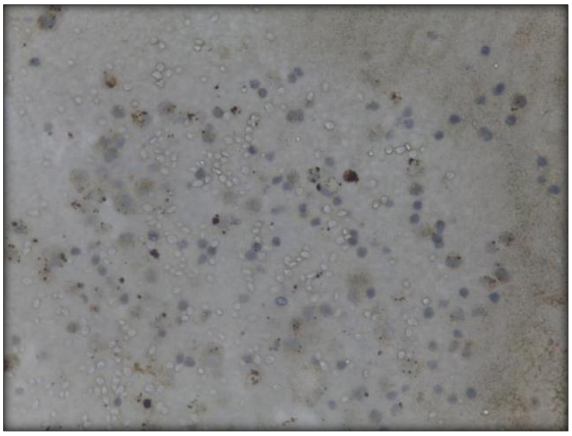
Calretinin negative in adenocarcinoma cells.



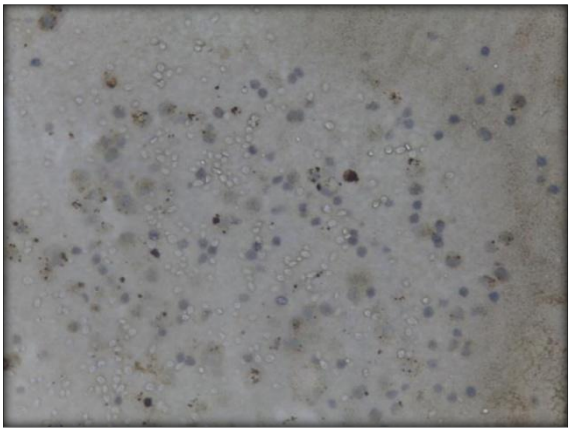
Reactive mesothelial cells in conventional smear (Pap stain)



Desmin show strong and diffuse cytoplasmic positivity. Staining score: 3; Intensity score: 3; IHC grade: 6



EMA negative in reactive mesothelial hyperplasia



Calretinin negative in reactive mesothelial cells.

Table 1:Basic characharestics of study population

Variables	N(%)
Age group	
21-40	3(5.4)
41-60	20(35.7)
61-80	29(51.8)
81-100	4(7.1)
Sex	
Male	17(30.4)
Female	39(69.6)
Colour of fluid	
Hemorrhagic	32(55)
Non-Hemorrhagic	24(45)
Distribution of primary organ in effusions	
Breast	4(7)
Colon	4(7)
Liver	2(4)
Lung	24(43)
Ovary	12(21)
Endometrium	2(4)
Stomach	5(9)
Unknown	3(5)

Table 2: Conventional smear, cell block and immunohistochemistry (IHC) comparison for final diagnosis

	Adenocarcinoma	Reactive Mesothelial Hyperplasia
Conventional Smear	23(41.1)	33(58.9)
Cell block	37(66.1)	19(33.9)
IHC	49(87.5)	7(12.5)

Table 3: Expression EMA and Desmin in effusions by immunohistochemistry

EMA Expression	
score<4	7(12)
4	4(7)
5	6(11)
6	39(70)
Expression of Desmin	
score<4	45(80.4)
4	4(7.1)
5	3(5.4)
6	4(7.1)

DISCUSSION

Pleural effusion cytology aids in diagnosing and managing malignancies but can be challenging in some cases. Ancillary techniques help differentiate between metastatic cancer, reactive mesothelial cells, and mesothelioma. This study aimed to distinguish malignant cells from mesothelial cells in pleural effusion using immunohistochemical markers (EMA, Desmin and Calretinin) on 56 cases diagnosed as reactive mesothelial hyperplasia or metastatic carcinoma .

The study analyzed 56 pleural fluid samples using smear preparation and cell block techniques. Patients ranged from 38 to 91 years, with the highest incidence (51.8%) in the 61–80 age group and the lowest in the 21–40 age group (5.4%). Pleural effusions were more common in females (69.6%) than males, with a male-to-female ratio of 1:2.1,

aligning with findings from previous studies.[7,8] Of the 56 effusions, 57% were hemorrhagic and 43% non-hemorrhagic. Malignancy was the leading cause of hemorrhagic effusions (87.5%), while 12.5% were reactive. Among 49 malignant effusions, 57.2% were hemorrhagic and 42.8% non-hemorrhagic.

In conventional smear cytology, moderate and marked cellularity were observed in 46.4% and 53.6% of cases, respectively, while in the cell block (CB) method, 80.3% showed moderate and 19.7% marked cellularity. About 43% of cases had better cellularity in CB, and 14.2% in conventional smears, with the remaining cases showing equal cellularity in both methods. CB also preserved morphological features, such as cytoplasm, cell membranes, and nuclear details, better than conventional smears, a finding consistent with previous studies.[9-11] Furthermore, CB demonstrated clearer architectural patterns, including papillae, glandular, and acinar structures. In distinguishing reactive mesothelial cells from malignant cells, CB was superior due to reduced nucleolar prominence and clearer structures, unlike conventional smears, which can mimic malignancy.[7,12,13] Out of the 56 pleural effusion samples, cytological diagnosis revealed benign effusions in 59% of the cases and malignancy in 41%. The sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) were 57.9%, 94.4%, 51.5%, and 95.6%, respectively. Malignancy was diagnosed in 66% of cases using the cell block method, with sensitivity of 77.5%, specificity of 100%, NPV of 38.8%, and PPV of 100%.The cell block method improves the detection of malignant cases, with diagnostic yields ranging from 15% to 25% higher than smears alone. It is particularly effective in detecting tumors in patients with negative or atypical cytology reports. Studies consistently show that CB outperforms smears in tumor cell recovery, with some studies reporting an increase in detection rates by up to 38%.[7,11]

The study found that the cell block method for diagnosing malignancy had a higher efficiency (75.5%) compared to the conventional smear method (46.9%), with a statistically significant difference (p - 0.018). Combining both tests improved diagnostic efficiency to 77.6%, which was significantly higher than the smear method alone (p - 0.001). Previous studies support these findings, with carcinoma of the lung being the most common primary neoplasm causing pleural effusion in both sexes, followed by ovarian cancer in females and gastric cancer in males. These results are consistent with those of other researchers.[14,15]

This study aimed to assess the utility of immunohistochemical markers (EMA, desmin, and calretinin) in differentiating reactive mesothelial hyperplasia from adenocarcinoma in serous effusions. The results showed that EMA was highly effective, with 78.7% of adenocarcinoma cases showing strong and diffuse cytoplasmic positivity. Combining EMA with cell block techniques increased the diagnostic yield by 21.4%, which was statistically significant. Desmin was found to be useful in identifying reactive mesothelial cells, though its sensitivity was lower compared to EMA. Calretinin was negative in all cases, including adenocarcinoma and reactive mesothelial hyperplasia, indicating limited value in this study's context. The study confirms that no single marker can reliably distinguish between benign and malignant cells with 100% accuracy. However, the combination of EMA and desmin proved to be the most effective, with EMA positivity and desmin negativity showing the best specificity for adenocarcinoma. This highlights the importance of using a panel of markers, rather than relying on a single one, to improve diagnostic accuracy, especially given the heterogeneity of metastatic malignancy.[16-20]

CONCLUSION

This study evaluates the diagnostic efficacy of Calretinin, Desmin, and EMA in distinguishing mesothelial cells from malignant cells in pleural effusions. The study found that combining conventional smear and cell block methods improved diagnostic yield, with cell block preparation offering better cellular details. IHC analysis identified 49 malignant cases, with EMA showing 100% sensitivity and specificity. Desmin was effective in identifying reactive mesothelial cells. Overall, combining EMA and Desmin enhanced diagnostic accuracy, making them a reliable panel for challenging effusion cases.

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