

Original research article**A cytomorphological study of serous effusions by various combined cytotechniques-conventional smear, cytospin and cell block**

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Abstract

Introduction: Cytological examination of serous fluids is one of the commonly performed investigation. It is crucial to distinguish between benign and malignant effusions, as it is important for determining the prognosis and treatment of the patient. Diagnostic problem arises in everyday practice to differentiate reactive atypical mesothelial cells from malignant cells by routine conventional smear method. Cyto-centrifuge will provide better quality smears for interpretation and cell block technique provides better architectural pattern, morphological features and an additional yield of malignant cells, and thereby, increasing the sensitivity of the cytodiagnosis when compared to conventional smears.

Aim: To compare the cytomorphological features of aspirates from body cavities using conventional smear, cytospin, cell block technique and perform IHC stains on cell block material in the diagnosis of malignant serous effusion.

Materials and Methods: A total of 262 serous fluid samples over a period of two years, were subjected to simultaneous processing by conventional smear, cyto-centrifuge and cell block technique. Results were compared for general cytological, cellular features and diagnostic utility for malignancy.

Results: Samples comprised of 172 pleural, 82 peritoneal and 8 pericardial effusions.

Conventional smear and cell block provided significantly better staining quality and morphological features. Cell block and cytospin cytology provided significantly high cellularity. Minimal overlapping of cells were significantly seen in cyto-centrifuge smears. Additional yield for malignancy was 4% more by cell block method.

Conclusion: The cell block technique not only increased the positive results for malignancy, but also helped to demonstrate better architectural patterns, which could be of great help in making correct diagnosis of the primary site. The cell block technique was also useful for special stains and immunohistochemistry. Cell block with ancillary technique like immunohistochemistry enhanced diagnostic accuracy.

Keywords: Cytomorphological, cytotechniques-conventional smear, cytospin and cell block

Introduction

Serous effusion fluids include pleural, peritoneal and pericardial fluids. Cytological examination of serous fluids is one of the commonly performed investigations. Cytology of body cavity fluids is an important tool for the diagnosis of malignancies, especially when other diagnostic tests cannot be performed on the patient. Fluid provides a more representative samples ^[1].

The detection of malignant cells in effusion fluids, besides implying the presence of cancer, also indicates the upstaging of the malignant disease. It is crucial to distinguish between the benign and malignant effusions, as it is important for determining the prognosis and treatment of the patients ^[2].

The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. Many studies suggest that cyto-centrifuge and liquid based cytology (LBC) provide better quality smears for interpretation and cell block technique provides better architectural pattern, morphological features and an additional yield of malignant cells and thereby, increasing the sensitivity of the cytodiagnosis when compared to conventional smears.

However, in many cases morphological features are not distinctive enough to distinguish mesothelioma from metastatic adenocarcinoma or a benign reactive effusion and it is necessary to perform immunohistochemical stains on cell block material.

Aims & Objectives of the study

1. To compare the cytomorphological features of aspirates from body cavities using conventional smear, cytospin and cell block technique.
2. To compare the diagnostic accuracy between cell block, cytospin technique and conventional smear cytology and perform IHC stains on cell block material in the diagnosis of malignant serous effusion. (With the aid of immunohistochemistry when needed).

Study sample size calculation: Assuming 85.7% sensitivity, 75% specificity the required sample size is 262 cases.

Study sample design: Comparative study.

Statistical of analysis: The results will be tabulated on daily basis in an excel sheet and analysed using SPSS software version 24. The data will be presented in terms of sensitivity, specificity, positive predictive value, negative predictive values. The diagnostic accuracy of cell block and cytospin will be assessed using appropriate statistical tools like Chi-square and Kappa test. Level of significance will be taken as 0.05.

Inclusion criteria

Serous effusions from the body cavities comprising of pleural, peritoneal and pericardial fluids received in pathology laboratory at AIMSR Hyderabad were included.

Exclusion criteria

Intraoperative peritoneal washings, synovial fluid, partially clotted samples and samples unfit for evaluation due to other technical reasons.

Results

This study on serous effusion samples was undertaken in the Pathology Laboratory and Department of Pathology, AIMSR, Hyderabad, over a period of 1 year from 1st August 2021 to 31st July 2022.

During this period total of 262 serous effusion samples were received for cytological examination. The following general observations were recorded.

Majority of the effusion samples were pleural in nature (65.6%), followed by peritoneal fluid (31.4%) and pericardial fluids (3%).

The maximum number of samples 77(18.8%) were from patients in 6th decade and least were from first decade. Male patients contributed to majority of the samples (M: F=2:1). In males, maximum numbers of samples were in age group of 51-60. In females, maximum number of samples was in age group of 61-70. Least number of samples was in the age group of 1-10.

On physical examination 52% of samples were clear in nature, where as 36% hemorrhagic and 12% turbid samples were respectively.

The maximum number of samples was exudative, 136 (52%) and total number of transudative was 126 (48%).

Each of the samples was subjected to the above modalities. (CS, CC & CB) and smears were assessed and compared, on the basis of parameters like staining quality, background, cellularity, architecture, cytomorphology and diagnostic utility for malignancy are compared.

The staining quality was “good” in 99.3% of CB, 97.1% of CS & 89.8% of CC.

The smear background was categorized as “clean”, “hemorrhagic”, “proteinaceous”, “mucoid” and “necrotic”. Majority of the fluids studied by CS show clean background followed by hemorrhagic and mucoid. Proteinaceous and necrotic background was seen in the least number of cases. A similar finding with regard to background was seen on CC and CB. The cellularity of the smear was interpreted as “high”, “moderate” and “low” based on the visual impression of the number of the mesothelial and malignant cells.

CB showed smears with predominantly high cellularity, while in CS and CC, the smears were predominantly of low and moderate cellularity respectively.

The architectural patterns like sheets, glandular patterns and papillary structures were more commonly observed in cell block followed by conventional smear, where as singly scattered cells were predominantly seen in CC.

The cytomorphology was studied in all the samples by observing the cytoplasm, nuclear margin, nucleus, nuclear cytoplasmic ratio and nucleolus of malignant cells, mesothelial cells, macrophages and other cells such as neutrophils, lymphocytes and eosinophils. All modalities showed good cytomorphology. The cytomorphological findings were consistently best observed on CB.

Out of 262 effusion samples, in CS cytological diagnosis of benign effusion was rendered in 240 (91.7%) cases, suspicious for malignancy in 14 (5.6%) cases and malignancy in 08 (2.6%) of cases.

In CC, 241 (92.2%) cases were diagnosed as benign, 15 (5.6%) cases as suspicious for malignancy and

06 (2.2%) cases as malignant.

In CB, 245 (93.7%) cases were diagnosed as benign, 17 (6.3%) as malignant. There were no diagnoses of suspicious for malignancy in cell block.

IHC was performed on cell block, in 10 cases to differentiate between reactive mesothelial hyperplasia and adenocarcinoma. The marker employed were Ber-EP4 and calretinin. Ber-EP4 positivity was seen in 8 of the cases, calretinin positivity in the 2 case was seen in the surrounding mesothelial cells and not malignant cell clusters.

On comparison with cell block, conventional smear has a sensitivity of 42.3% but specificity of 97.9%. Positive predictive value (PPV) was 57.9%, and negative predictive value (NPV) was 97.24%. Conventional smear had a low sensitivity but high specificity. The PPV was moderate but NPV was high. Discrepancy was noted in 28 cases in CS. Analysis of these 28 cases of effusion samples showed that five cases which were reported as benign effusions in conventional smear method were diagnosed as malignant lesions by cell block method. Out of these, two cases were reported as reactive mesothelial hyperplasia, three cases were misdiagnosed, as morphology was obscured by hemorrhagic background, plenty of inflammatory cells and reactive mesothelial cells. Suspicious for malignancy was reported in 23 cases on conventional smears, in that 10 cases were diagnosed as malignant effusions by cell block. By cell block method, additional 15 cases were detected as malignant, i.e. 3.65 % more diagnostic yield for malignancy. On comparison with cell block, cytocentrifuge has a sensitivity of 34.6% but specificity of 97.9%. Positive predictive value (PPV) was 52.9%, and negative predictive value (NPV) was 95.7%. Cytocentrifuge had a low sensitivity but high specificity. The PPV was moderate but NPV was high. Discrepancy was noted in 26 cases in CC. Analysis of these 26 cases of effusion samples showed that three cases which were reported as benign effusions in cytocentrifuge method were diagnosed as malignant lesions by cell block method. Three cases were misdiagnosed, as morphology was obscured by hemorrhagic background, plenty of inflammatory cells and reactive mesothelial cells. Suspicious for malignancy was reported in 23 cases, in that 14 cases were diagnosed as malignant effusions by cell block. By cell block method, additional 17 cases were detected as malignant, i.e. 4.14% more diagnostic yield for malignancy.

Of the 26 malignant effusions, 14 were transudative in nature and 12 were exudative.

Of the 26 malignant effusions, 14 (53.85%) were pleural effusion, 10 (38.46%) were peritoneal effusion and 2(7.70%) were pericardial effusion. Total number of 26 serous effusion samples was diagnosed as malignant effusion by cell block method which included 14 pleural, 10 peritoneal and two pericardial fluids. In all 26 cases diagnosis of metastatic malignant effusion was rendered. Out of 14 cases of malignant pleural effusions primary was known in 10 cases, which included four cases of carcinoma of lung, three cases of carcinoma of gastrointestinal tract, and one case each from carcinoma of breast, larynx and ovary. In remaining four cases primary could not be detected as patients were lost for follow up study.

In analysis of 10 cases of malignant peritoneal effusion primary were detected in nine cases. In five cases primary was in ovary, two cases primary was in colon and remaining two cases each were from carcinoma endometrium and carcinoma liver. In remaining one case, primary was not known, as patients lost for follow up study. Out of two cases of malignant pericardial effusions, one case was from carcinoma lung and primary of other case was not known.

The malignant effusions were more common in age group of 61-70 years. Out of 26 cases, 14 cases (54%) in females and 12 cases (46%) in males. Female to male ratio was 1.16:1 in malignant effusion. The commonest primary was identified in ovary followed by GIT and lung. Primary was unknown in 21% of cases.

Significance of difference of each parameter for four groups was performed using friedman anova test Significance of difference of each parameter for two groups was performed using Wilcoxon rank sum test.

Friedman anova test indicated statistically significant difference for each parameter ($p < 0.0001$) across four methods.

The two group comparison was performed using Wilcoxon rank sum test. It revealed conventional smear and cytocentrifuge, conventional smear and cell block, as well as cytocentrifuge and cell block on each parameter were statistically significant ($p < 0.05$), except for the background, which was statistically insignificant ($p > 0.05$).

Discussion

The cytological examination of serous effusions has increasingly gained acceptance in clinical medicine, to such an extent that a positive diagnosis is often considered the definitive test and obviates explorative surgery. It is important not only in the diagnosis of malignant lesions, but also help in staging and prognosis^[1, 10, 15].

In most of the cytology laboratories, cytologists prefer direct smear prepared from centrifuged deposits of effusion. The cell block technique which is amongst the oldest method of processing cytological material for microscopy has been abandoned by many laboratories as several alternative excellent cell

preparation methods have been developed. However, several authors have reported the advantages of cell blocks in cytology which includes valuable diagnostic evidence that can be observed in smears^[10, 15]. Lack of morphological details of the representative cells contributes to considerable difficulties in making conclusive diagnosis on conventional smears. In order to overcome these difficulties, in this study an attempt was made to prepare and analyse routine centrifuge, cytocentrifuge, liquid based cytology and cell block from the same sample. In this study due consideration was given to age, sex, site of effusion, clinical findings and investigations to arrive at final diagnosis and also to identify primary malignant lesion.

We received 410 samples of body cavity fluids of which pleural fluid samples contributed more i.e. 67.6%. Peritoneal and pericardial fluid samples were 31.5%, and 1% respectively. Similar findings were noted by Dadhich *et al.* study in which pleural fluid samples were 52%, peritoneal samples were 44% and pericardial were 4%^[7].

The age of the patients included in our study ranged from 10 to 90 years, with a male preponderance (M:F=2:1). Maximum samples were belonging to age group of 51-60. Least of samples were from age group of 1-10. In females the most common age group was seventh decade and in males it was sixth decade. Similar age and sex distribution is noted in other studies from India^[13, 14].

Staining quality was good in all the three techniques except for liquid based cytology smears, in which yielded 77.6% of good smears, which is comparable with the findings of study by Dadhich *et al.* Liquid based cytology smears showed a clearer background in majority of cases (91%), by removing obscuring materials such as blood, inflammatory cells, proteinaceous and cellular debris, in contrast to other three modalities, with a statistically significant difference (P value of <0.001). Similar findings were reported in other studies as well^[7].

Majority of liquid based cytology smears exhibited a less cellular central area with a more cellular peripheral rim. Similarly, Choi *et al.*^[16] reported 67 of 474 cases exhibited more cells in the periphery of the slide. Consequently, they concluded that cytological diagnosis based on cells in the peripheral area should be made with caution, since the peripheral compression artefact affects the size of cells and their degree of chromatin compaction, which are key criteria in diagnosis of malignant cells.

Smears were highly cellular in liquid-based cytology and cell block smears, which is comparable with Dadhich *et al.*^[7] and Udasimath *et al.*^[8] However, a study of Alwahaibi *et al.*^[17] found conventional smear to be highly cellular compared to smears prepared by liquid-based cytology, which they attributed to the use of split samples and there are some studies showing high cellularity with the conventional smears^[18].

Few studies have shown increased cellularity with cytocentrifuge technique^[10, 15].

But in present study majority of cytocentrifuge smears were moderately cellular.

In comparison with other three modalities, liquid based cytology smears showed an even distribution of cells forming monolayer with minimal overlapping.

Even cytocentrifuge preparations, reduced the overlapping of cells, enabling precise interpretation than conventional smears. These findings are consistent with the findings in the study done by Alwahaibi *et al.* and Joshi *et al.*^[10, 17].

In addition to increased cellularity, cell block method provided a better morphological detail, like architectural patterns (three dimensional clusters), with better nuclear and cytoplasmic preservation and intact cell membrane. Glandular or acinar structures and nucleoli were better appreciated in cell block study when compared to other three techniques. The reactive or atypical mesothelial cell which simulate malignancy in smears of other modalities were identified confidently as reactive or mesothelial cells by cell block method. Similar findings were noted in various studies^[8, 9, 12, 13].

The cytomorphology of the smears studied was shown to be better in cell block, cytocentrifuge and conventional smears than liquid based cytology smears with a P value of <0.001 which was statistically significant. Many studies have shown no significant difference between the methods in the cytomorphology of cells^[19, 20].

Although, there are also studies claiming a better preservation of cells with good cytomorphology in liquid-based cytology smears^[6, 17, 21].

Cells in liquid-based cytology in present study appeared smaller and pleomorphism was not as striking as was in conventional and cytocentrifuge smears.

This observation has been also noted by Dadhich *et al.*^[7] The smaller cell size in liquid-based cytology is due to cell shrinkage effect of liquid based cytology preservative, while the larger or more pleomorphic appearance of cells in conventional and cytocentrifuge smears is attributed to nuclear swelling in the air-dried smears.

Comparison of cytodiagnosis of serous effusion in present study with other studies

Many studies were conducted to compare cytodiagnosis of conventional smear and cell block technique. Additional yield of malignancy by cell block in this study was 4% which was less than that of studies done by Thapar *et al.*^[9] and Udasmith *et al.*^[8], in those studies additional yield of malignancy by cell

block was 13% and 15% respectively. Reason for the low yield of malignancy in this study may be due to technical errors such as inadequate sampling or degenerated samples.

A study titled as usefulness of cell block versus smears in malignant effusion cases by Khan *et al.*, reported that the recovery rate for malignant lesion by cell block preparation was 20% greater than that obtained for specimen examined in smear only [13].

According to various studies additional diagnostic yield for malignancy was noted in conventional smear technique is supplemented by cell block method [13,22].

In a study conducted by Joshi *et al.* [10] and Singh *et al.* [15], compared cytodiagnosis between conventional smear, cytocentrifuge and cell block. The major difference in cytodiagnosis among the three groups in these studies was in the suspicious category.

On conventional smears few cases were diagnosed as suspicious, where as not a single case diagnosed as suspicious in cytocentrifuge and cell block. Yield of malignant effusion diagnosis increased with cytocentrifuge and cell block. Difference between cytocentrifuge and cell block in diagnosis of malignant effusion was statistically insignificant in these studies, [10, 15] which is discordance with present study.

In present study same number of cases were reported as suspicious on conventional as well as in cytocentrifuge smears. There is statistically significant difference between results obtained by cytocentrifuge and cell block technique in diagnosis of malignant effusion.

Leung *et al.* [19] and Dadhich *et al.* [7], compared cytodiagnosis between conventional smears and liquid based cytology smears. No significant difference was observed in the diagnostic categories between conventional smear and the liquid-based cytology. This statistics showed a very good agreement between conventional smear and liquid based cytology in recognising malignant cells in the smears, which is discordance with other studies that have claimed a higher detection rate of malignant cells with liquid based cytology.

Present study is in concordance with that of Leung *et al.* [19] and Dadhich *et al.* [7] who noted no statistically significant difference.

In a study conducted by Yim *et al.* [23] and Mittal *et al.* [24], compared cytodiagnosis between conventional smear, liquid based cytology and cell block technique. They noted additional yield for malignancy by liquid-based cytology was more compared to conventional smear and cell block methods in diagnosing malignant lesion, which is discordance in present study [23, 24].

In present study by cell block method additional 19 cases which were suspicious in liquid-based cytology smears were detected as malignant i.e. 4.63% more diagnostic yield for malignancy by cell block method. This difference might be due to that liquid-based cytology was not standardised yet for non gynaecologic samples.

Total of 26 serous effusion samples were diagnosed as malignant effusion by cell block method in this study which included 14 pleural, 10 peritoneal and two pericardial fluids. All 26 cases were metastatic malignant effusion. Most of the tumors were of adenocarcinoma type.

Carcinoma of lung was the commonest site followed by carcinoma of ovary and carcinoma of GIT in a study conducted by Khan *et al.* [13]. Similarly Murphy *et al.* [4] described that the commonest primary malignant lesions were in breast followed by lung and ovary. Sears and Hajdu *et al.* [3], reported that the most common primary neoplasms causing pleural effusions were carcinoma of breast (24%), followed by lung (19%) and lymphoreticular system (16%), where s primary, in peritoneal effusions were ovary (32%), breast (15%) and lymphoreticular system (7%). In 15% of cases primary site was unknown.

Carcinoma of lung was the commonest primary followed by primary in GIT, ovary and breast in pleural effusions in this study. In peritoneal effusions ovarian carcinoma was the commonest primary followed by carcinoma of GIT, liver and endometrium. Similar pattern of primary lesions were reported by Bonito *et al.* [25].

We noted the presence of pericellular lacunae in more than 60% cases of adenocarcinoma, especially of mucin secreting type, characterised by large cell clusters. Prince *et al.* [5] and Udasimath *et al.* [8] also revealed the presence of pericellular lacunae especially in cases of adenocarcinoma samples.

Reactive mesothelial cells can at times show cytologic features indistinguishable from cells shed from an adenocarcinoma leading to erroneous diagnosis.

Monoclonal antibodies against various mesothelial and epithelial antigens have been used to aid in differentiating the two. Over the past several years, various studies have analysed the efficacy of a variety of antibodies for this purpose [11, 26, 27].

But till date, there has been no consensus on the panel of antibodies to be used. Some authors have recommended single antibodies, mostly calretinin [28, 29, 30].

We do not agree with the use of single monoclonal antibody for this purpose, which has diagnostic as well as prognostic implications. This is in agreement with other authors [31, 32].

A minimum of two antibodies, a mesothelial and an epithelial marker, should be included. We analysed two monoclonal antibodies i.e. calretinin (a mesothelial marker) and Ber-EP4 (an epithelial marker) on cell block sections on cases which were difficult to differentiate papillary reactive mesothelial hyperplasia from adenocarcinoma. Four out of five cases showed Ber-EP4 positivity and one case showed calretinin positivity. Similar findings were observed in studies conducted by other author's also

[31, 32, 33]

Conclusion

The value of cytological examination of serous effusions is widely recognized and well documented. The primary role of cytology in this setting is detecting malignancy. Detailed cytomorphological features of various metastatic malignant cells in effusions provide definitive clues regarding the primary site. The diagnostic performance of cytological study of the fluid may be attributable to the fact that the cell population present in the sediment is representative of a much larger surface area than that obtained by a needle biopsy.

Routine conventional smear is not satisfactory in reporting fluids with scant cellularity. Hence for fluids with scant cellularity cytocentrifuge and cell block are useful methods. The morphological interpretation of liquid based cytology preparation remains a challenge because of somewhat altered morphology and artifacts or facts resulting from the preservative, fixation and processing techniques. These changes include architectural changes such as smaller cell clusters and sheets, breakage of papillae, altered cell distribution with more dyscohesion and changes in cellular morphology with enhanced nuclear features and smaller cell size. This study establishes the superiority of cytopspin over other two modalities with regard to clearer smear background in serous body fluid cytology.

However, with regard to the staining quality and morphologic assessment of various cellular components, the conventional smear and cell block technique continues to remain a preferable tool, thus aids in accurate diagnosis.

Diagnosis which were missed or suspicious on conventional smear and cytocentrifuge were diagnosed accurately by the cell block technique.

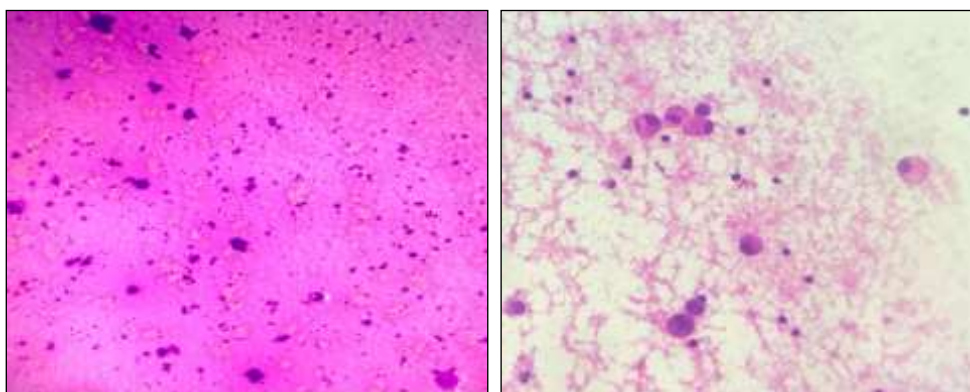
Cell block technique is simple, inexpensive and does not require any special training or instrument. Cell block yielded more cellularity with better architectural preservation than other three modalities. Multiple sections can be obtained for special stains or immunohistochemistry study. Sensitivity for malignant lesion by cell block was significantly increased compared to other modalities. Thus, cell block proved to be superior method for the study of effusion as compared to other cytopreparatory techniques.

A limited panel of two monoclonal antibodies, calretinin (mesothelial marker) and Ber-EP4 (epithelial marker), to differentiate reactive mesothelial hyperplasia from adenocarcinoma may be useful in cytology as a “primary antibody panel”, for accurate diagnosis and patient management.

Though conventional smear most popular technique used when comes to diagnosis, better techniques may be adopted depending on availability of sources in the laboratory. Cell block study with immunohistochemistry for definitive diagnosis.

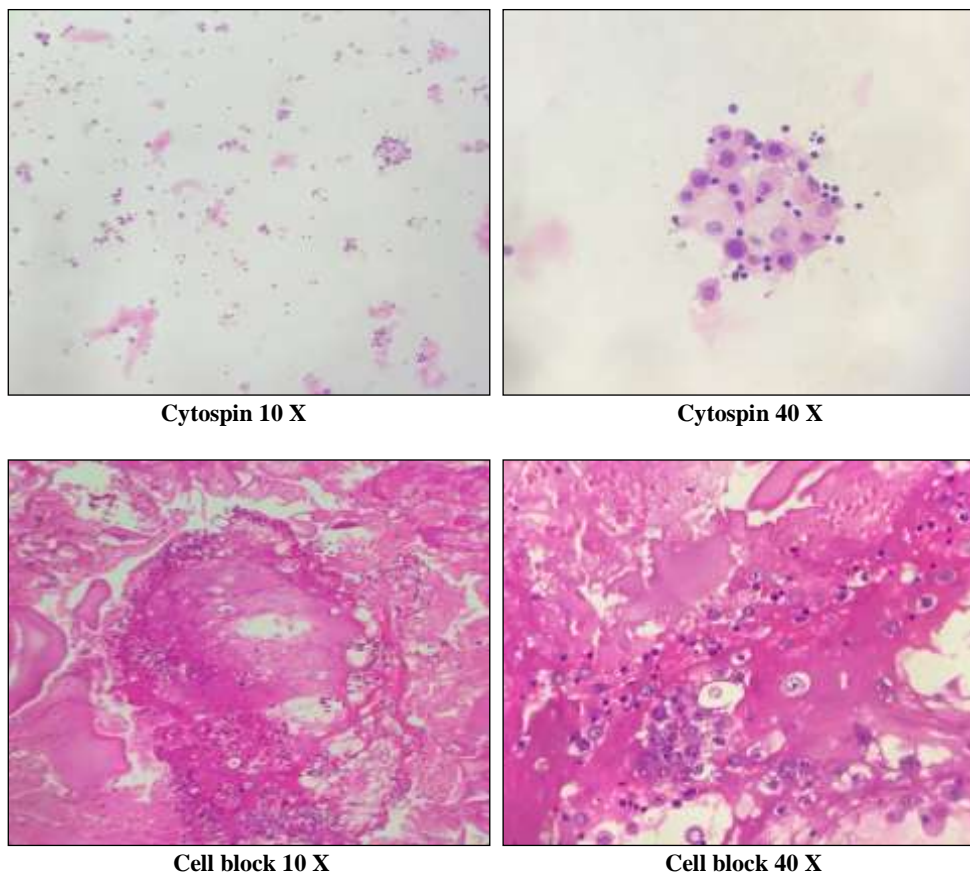
Statistical analysis revealed low sensitivity and high specificity of three modalities compared to cell block in diagnosing malignant effusions. It also revealed highly significant difference between all the four modalities.

- Cell block showed high sensitivity and specificity in diagnosis of malignant effusion.
- Thus utility of cell block method in cytodiagnosis of malignant effusion is highly significant as compared with other three techniques.



Conventional smear 10 X

Conventional smear 40 X



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