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Original Research

Comparative Study Of Conventional Smear And Cell Block In The Cytodiagnosis Of Pleural And Peritoneal Effusion

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ABSTRACT

BACKGROUND: This study was conducted tocompare studies of pleural and peritoneal effusion, assess the quality and diagnostic features between conventional and cell block preparation and evaluate the role of cell block to be used along with conventional smear for routine cytological practice.

METHODS: This was a hospital-based cross-sectional study conducted in tertiary care center among 168 samples to compare conventional smears with cell block preparation in increasing the sensitivity of cytodiagnosis of pleural and peritoneal fluids received in the cytology laboratory at the Department of Pathology, Tertiary Care Hospital over the period of two years.

RESULTS: In contrast to just 3.6% in traditional smears, 46.4% of instances displayed extensive cellularity on cell blocks. In cell blocks, the proportion of instances exhibiting minimal or nonexistent cellularity drops from 95% in conventional smears to 3%. While the percentage of cells with appropriate cellularity was lower in cell block (50.6%) than in conventional smear (86.9%), the statistical difference was still significant. The mean value of different cytological parameters in the CB is comparatively higher than in CS and this difference is found to be significant statistically. The frequency of malignant cells detected by the cell block technique was higher than that of the conventional smear (n = 14 versus n = 8), and this difference was also found to be statistically significant when comparing the two cytodiagnostic methods for the final diagnosis of specimens.

CONCLUSION: To sum up, a combination of the cell block technique and conventional smears should be utilized not just for questionable effusions on conventional smears but also as a standard procedure for all effusions received in order to uncover concealed cases of cancer or other diseases. For labs with low resources that can manufacture cell blocks utilizing plasma thromboplastin in an economical manner, this method is ideal.

KEYWORDS: Conventional Smear, Cell Block, Cytodiagnosis, Pleural and Peritoneal Effusion.

INTRODUCTION

Pleural cavity is a potential space between parietal and visceral pleura; normally some amount of fluid is present in the pleural cavity. Pleural fluid is produced by the cells of the parietal lining and absorbed by the visceral lining'.[1] The peritoneal cavity is the largest cavity in the body, lined by flattened polyhedral cells (mesothelial cells). Normally only a few millimeters of peritoneal fluid are found in the peritoneal cavity. This fluid facilitates the movements of two membranes against each other.[2] The amount of fluid in these cavities is increased (pleural effusion and ascites) in certain disease processes like inflammation and neoplasm.[3]

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Effusions are classified into two types, transudates or exudates, by using Lights criteria, which are as **follows:**

(1) Serous fluid protein/serum protein>0.5,
(2) Serous fluid LDH/serum LDH>0.6,
(3) Serous fluid LDH > 2/3 of the upper limit of normal for serum LDH.

Exudates have at least one of the above criteria. The presence of all three criteria best differentiates exudates from transudates. Transudates have none of these criteria.[4]

Cytological examination of serous fluid by preparing conventional smears has been used for diagnosis as they are simple to prepare, inexpensive, and do not involve any invasive procedure. Despite these advantages, examination of serous fluid has lost its popularity due to its lower sensitivity, which is attributed to the loss of morphological details of cells, overcrowding or overlapping of cells, and loss of cells during processing. In serous effusions, accurately classifying cells as either benign reactive mesothelial cells or malignant cells is a frequent diagnostic challenge. Pathologists find it challenging to differentiate between these two.[5]Numerous investigations have shown that even when fluid is cytologically examined using smears, a significant residue is left behind that is not further examined yet may contain useful diagnostic information. By treating this leftover material as a cell block, embedding it in paraffin, and examining it in addition to the standard smears, it can be assessed quickly and easily.[6,7]

When cytological abnormalities are unclear, as in occasionally well-differentiated adenocarcinomas, or misleading, as in reactive mesothelial cells, cell blocks are very helpful.

It has multiple advantages, such as:

- Better morphology of cells and architecture (especially in neoplastic lesions).
- Scope for multiple sections for special stains and other ancillary studies like immunohistochemistry, etc.
- Preservation of architectural patterns like cell balls, papillae, and three-dimensional clusters.
- Intact cell membranes and crisp chromatin details.
- There is adequate cellularity and delineation of the nucleus and cytoplasmic details.
- Loose cells, cell aggregates, and microscopic tissue fragments are easily recoverable.
- It bridges the gap between cytology and histology.
- Concentration of cellular material in one small area that can be evaluated at a glance with all cells lying in the same focal plane.[6]

In order to increase the sensitivity of cytodiagnosis of pleural and peritoneal fluids obtained in our cytology laboratory, the current study was designed to compare traditional smears with cell block preparation.

Aims and Objectives

- To compare studies of pleural and peritoneal effusion and to assess the quality and diagnostic features between conventional and cell block preparation.
- To evaluate the role of cell block to be used along with conventional smear for routine cytological practice.

METHODS

This was a hospital-based cross-sectional study conducted among 168 samples to compare conventional smears with cell block preparation in increasing the sensitivity of cytodiagnosis of pleural and peritoneal fluids received in the cytology laboratory at the Department of Pathology, Tertiary Care Hospital over the period of two years.

Statistical Methods

The final data was tabulated and statistical tests were applied. The Mc Nemar chi square test was used to calculate the p-value. The p-value was calculated under the predetermined level of significance of 0.001.

RESULTS

Diagnostic Cellularity	Point Score	Conventional Smear	Cell Block	P-Value
Minimal/Absent	**0**	**16 (95%)**	**5 (3%)**	**0.001**
Sufficient	1	146 (86.9%)	85 (50.6%)	0.001 (chi sq value is 59.01)
Abundant	2	6 (3.6%)	78 (46.4%)	0.001 (chi sq value is 70.01)
Total		**168 (100.0%)**	**168 (100.0%)**	

Table 1: Comparison of Diagnostic Cellularity in the Conventional Smear and Cell block Techniques

In contrast to just 3.6% in traditional smears, 46.4% of instances displayed extensive cellularity on cell blocks. In cell blocks, the proportion of instances exhibiting minimal or nonexistent cellularity drops from 95% in conventional smears to 3%. The difference was statistically significant even if the percentage of findings of appropriate cellularity is relatively lower in cell block (50.6%) than in conventional smear (86.9%).

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Parameters	CS	CB	T-Value	P-Value
Diagnostic Cellularity	0.94 ± 0.36	1.43 ± 0.55	-12.770	0.001
Obscuring Blood	1.24 ± 0.45	1.63 ± 0.49	-9.819	0.001
Cell Architecture	0.97 ± 0.18	1.23 0.43	-7.471	0.001
Cell Degeneration	0.97 ± 0.17	1.03 0.17	-3.251	0.001
Table 2 : Comparison of Different Cytological Parameters in the CS and CB Techniques				
***Paired student's t test**				

The mean value of different cytological parameters in the CB was comparatively higher than in the CS and this difference was found to be significant statistically.

Final Diagnosis of Specimen	Conventional Smear	Cell Block	P-Value
Benign	154	154	Not calculated
Malignant	8	14	0.031
Suspicious	6	0	Not calculated
Total	168	168	
Table 3:Comparison of Conventional Smear and Cell Block Cytodiagnostic Techniques in the Final Diagnosis of Specimens			

When using the cell block technique, the frequency of malignant cells is higher than when using a traditional smear (n = 14 versus n = 8), and this difference was also determined to be statistically significant. In the portions of the cell block, the six instances that were identified as suggestive of malignancy on a conventional smear were found to be malignant. Since nuclear and cytoplasmic features were unclear and vacuolations with eccentric nuclei could be recognized even in a degenerating and/or reactive mesothelial cell in a conventional smear, they were categorized as suggestive for malignancy (n = 6). One such case showing prominent vacuolation with indistinct nuclear details was classified as suspicious for malignancy. Better architectural preservation with the formation of cell balls, acini, and glandular structures along with morphological preservation with better cytoplasmic as well as nuclear details, crisp nuclear chromatin, the presence of nucleoli and clear nuclear margins in cell blocks were helpful features in differentiating malignant cells from reactive mesothelial cells. The number of benign cases found on both conventional smears and cell blocks remains the same, i.e., n = 154. Therefore, in our study, cell blocks proved to be beneficial to confirm the diagnosis of malignant cases and to aid in making a diagnosis of malignancy in suspicious cases.

DISCUSSION

One method for identifying the existence of cancer in bodily fluids is cytological examination. Numerous research' findings attest to the superiority of fluid examination in cancer diagnosis.[8] In clinical practice, cytological analysis of serous fluids has become so commonplace that a positive result is frequently regarded as the gold standard, even negating the need for exploratory surgery. The severity of the illness and the type of primary cancer determine the diagnostic yield. There are numerous methods for evaluating fluid that has been submitted for cytological analysis. For this aim, routine cytological smears are preferred by the majority of laboratories.The sensitivity, specificity, efficiency, and positive and negative predictive values of smears were 44.55%, 95.7%, 50.1%, 98.7%, and 20%, respectively, according to a study by Oyafuso et al.[9] Mother et al., also reported similar findings.[10] These results unequivocally demonstrate that conventional smears used in effusion cytology do not provide adequate diagnostic accuracy; as a result, adjuvant techniques were required to diagnose serous fluids. The inability to differentiate reactive mesothelial cells from metastatic malignant cells is one of the most prevalent and upsetting limitations of traditional smears. This is either because of the subtle cytomorphological characteristics of some malignant neoplasms, especially well-differentiated adenocarcinomas, or because of the noticeable atypia of mesothelial cells brought on by microbiological, chemical, physical, immunological, or metabolic insults to the serous membrane as a result of the disease process.[11] The cell block technique has been widely utilized in fluid processing ever since Bahrenburg first introduced it. Even in environments with limited resources, the method is easy to use, safe, economical, and repeatable. With reduced background staining and results that most closely resemble those published in the surgical literature, cell blocks offer the greatest milicufor morphologic interpretation.[12]

Since original malignancies originating from mesothelial cells are rather rare, the presence of malignant cells in pleural and ascitic fluid is virtually always a sign of metastatic tumors. One crucial prognostic factor for these patients is a positive effusion of cancer cells. An advanced stage of cancer is indicated by the formation of a malignant effusion. Therefore, it is now commonly accepted as a regular laboratory method for the diagnosis of metastasis from an unknown primary origin that involves examining serous fluids for the presence of malignant cells in an effusion.[5]

In this study, an attempt was made to prepare and analyze both smears and cell blocks from the same specimen. Due consideration was given to age, sex,

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site of effusion, and clinical and radiological findings to arrive at the final diagnosis. We assessed the cellularity, architectural pattern, prominent cells, amount of concealing blood, and morphological preservation of both traditional smears and cell blocks.

In the present study, the cell block preparation was done by the plasma thromboplastin method, as this uses outdated plasma from the blood bank and thromboplastin reagent from the hematology laboratory, making this a cost-effective method. The advantages are that it is reproducible, concentrates more cellular material, and forms more solid cell buttons due to the formation of clots and better cellular preservation. Shukla P et al.,[13] and Kulkarni et al.,[14] have used this method for cell block preparation. Bodele et al.,[15] Thapar et al.,[5] and Shivkumarswami et al.,[11] have used the sediment method with 10% alcohol and formalin as fixatives, while Sujathan et al.,[16] have used the sediment method with ethanol, acetic acid, and formalin as fixatives.

A total of 168 specimens were studied, which comprised of 131 (78%) pleural and 37 (22%) peritoneal effusions. Therefore, the number of pleural fluids was much greater than peritoneal. Our results are similar to Subhada et al.,[17]Bhanvadia et al.,[12] Nair et al.,[8] and Shobha et al.,[18] However, Sujathan et al.,[16] Joshi et al.,[19] and Nathani et al., have reported a larger number of peritonial fluids in their study.

There was a male preponderance seen among the patients, with the male:female ratio being 1.27:1. Similar results were seen by Nair et al., (M:F=1.55:1) and Joshi et al.,[19] (M:F-1.05:1). In pleural effusions alone, males outnumbered the females (M-87, F-44) in our study. Bhanvadia et al.,[12] (M-61, F-18) and Shivkumarswamy et al.,[11] have also shown the same. Peritoneal effusions, on the other hand, had a female preponderance in our study, which is similar to what was seen by Bhanvadia et al.,[12] but contrary to the results reported by Pal et al., in their study, where a male preponderance was noted (M:F-1.5:1).

The present study showed more exudative effusions (n-144) than transudative (n-24). However, in the study conducted by Bhanvadia et al., the number of transudative effusions (n = 91) was much higher than exudative effusions (n = 59).

Regarding the age distribution of patients in our study, Pal et al., have also reported the maximum number of cases in the same age group, i.e., in the 40-60 age group collectively.

As far as the provisional clinical diagnosis was concerned, in reactive effusions, tuberculosis was the commonest, accounting for 58.3% of the cases. Thapar et al.,[5]have also shown a similar trend, reporting 18.3% of the effusions where the underlying pathology was tuberculosis. Joshi et al.,[19] Shubhada et al.,[17] and Shukla et al.,[13] (33%) have also seen similar results in their study, where the major proportion of non-neoplastic effusions was due to tubercular pathology. Shobha et al.,[18] have also reported the maximum cases of reactive pleural effusion (52%) having tubercular etiology.

Nair et al., also reported a similar result in pleural fluid, but in peritoneal fluids, cirrhosis was the commonest cause, followed by tuberculosis. Nathani et al. had maximum cases of cirrhosis followed by congestive cardiac failure and then tuberculosis as the cause of serous effusions in their study. However, Luse and Reagan[20] reported underlying congestive cardiac failure as the cause of maximum effusions in their study. This difference may be largely due to the difference in the region where the respective studies were carried out. Studies that are conducted in India and the neighboring countries, which share almost the same geographical terrain and climatic conditions, also share common endemic trends for certain diseases, especially tuberculosis.

In reactive effusions, we found lymphocytes as the predominant cell in maximum cases (67.5%), whereas Thapar et al.,[5]reported polymorphs as the predominant cell (21.7%) in maximum cases.

On evaluating the cellularity of the smears, score 0 (CSO) was observed in 95%, score 1 (CS1) in 86.9%, and score 2 (CS2) in 3.6%, whereas score 1 (CB0) was 5%, score 2 (CB1) was 50.6%, and score 3 (CB2) was 46.4% in cell blocks. These results clearly depict the superiority of the cell block technique over conventional smears. Shukla et al. and Shubhada et al. have also shown a definite advantage that cell blocks have over smears in the cellular yield.

When assessment for retention of architecture was performed, score 0 was observed in 3% smears which reduced to 0% in blocks; score 1 was 96.4% in smears and 76.2% in blocks; and score 2 increased from 0.6% to 23.8% in cell blocks. Shukla et al.[13] have results in congruence with our study: score 2, which increased from 20% in smears to 40% in cell blocks. Shubhada et al. also report an improvement in retention of architecture in cell blocks in comparison with smears; score increased from 0.7% in smears to 11.27% in blocks, and score 2 showed a rise from 0% to 9.86% in cell blocks. Cell block preparations revealed better cytoplasmic and nuclear details as compared to conventional smears.

On finally assigning the diagnostic categories to the smears and cell block preparations, our study showed that the diagnostically unsuitable category was reduced from 6.57% to 0.6% in blocks and diagnostically superior was raised from 0.6% to 50%, thereby leaving no doubt on the superiority of the cell block technique over conventional smears. Nathani et al., and Thapar et al.,[3] also show compatible results in their respective studies.

By using the cell block technique, we obtained an additional diagnostic yield of 42.8%. Similar studies conducted over the years have also reported a better diagnostic yield by cell blocks.

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This additional diagnostic yield in cell blocks, especially in malignant effusions can be explained. In contrast to a normal smear, where the cells stay distributed and there are few representative cells, a cell block consolidated the cellular material into a compact region, making it easier to screen the material in less time with cells laying in the same focus plane.[21,22] It is difficult to distinguish between reactive mesothelial cells and metastatic neoplasms in conventional smears.

This is usually due to marked atypia of mesothelial cells due to various insults over serous membranes. Besides, malignant cells from well-differentiated adenocarcinomas may show only subtle cytomorphological features similar to normal mesothelial cells, such as tumors of the breast, lung, or gastrointestinal tract.[23] In conventional smears, degenerating mesothelial cells can occasionally look as signet ring cells with huge vacuoles and eccentric nuclei, which can be mistaken for tumors secreting mucin. They might also have noticeable nucleoli. Cell blocks effectively place both architectural (rossetes, pseudoacini, or acini) and morphological (prominent nucleoli) features in their proper perspective, making it easier to distinguish between reactive mesothelial cells and malignant cells. This improved the diagnostic yield for malignant effusions. Acinar structures are better seen when present in cell blocks, and nucleoli are less noticeable in traditional smears. The cytomorphological and malignant character of both cells in well-differentiated adenocarcinomas are better observed in cell blocks that contain genuine acini.[6,11]

Similar to the results of Bhanvadia et al., gaps and windows in reactive mesothelial cells were commonly seen in the current investigation.

Cell blocks, as opposed to traditional smears, provided a more reliable view of the glandular structures, papillary structures, clusters, 3D balls, and the presence of mucin in the cytoplasm of tumor cells. Besides this, nuclear and cytoplasmic details were sharp and distinct, which is in agreement with studies conducted by Thapar et al.,[5]Bhanvadia et al.,[12] Dekker et al.,[6]Shivkumarswamy et al., Joshi A et al.,[19] Shukla P et al.,[13] noted target inclusions in adenocarcinoma, which were however absent in the present study.

There was no extra diagnostic yield in reactive effusions when cell blocks were prepared as opposed to conventional smears in this investigation, although preservation of the architecture and morphology of cells in reactive effusions was also significantly better in cell block preparations than in conventional smears. Additionally, Shobha SN et al.[18] showed that reactive effusions obtained via cell block based on architectural and morphological preservation did not offer any useful diagnostic value. However, the presence of well-formed granulomas in cell blocks allowed them to make a definite diagnosis of tuberculosis in four cases with reactive effusions.

Thus, in labs with limited resources, the cell block technique offers an economical approach. It improves architecture display and morphological preservation in addition to improving cellularity, which raises the diagnostic yield. In serous fluid cytology, it should be utilized in addition to traditional smears to help and enhance the diagnosis.

CONCLUSION

With reduced cellular dispersal and the benefit of several sections on which specific stains can be put, cell blocks can be studied in a biopsy-like manner. Additionally, slides and blocks may be stored for a longer period of time. In addition, sections from cell blocks improve cellularity with less blood obscuring, better preserve architecture, and have great cytoplasmic and nuclear features, all of which raise the diagnostic yield. To sum up, a combination of the cell block technique and conventional smears should be utilized not just for questionable effusions on conventional smears but also as a standard procedure for all effusions received in order to uncover concealed cases of cancer or other diseases. For labs with low resources that can manufacture cell blocks utilizing plasma thromboplastin in an economical manner, this method is ideal.

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