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Efficacy of Cell Block Technique in the Cytodiagnosis of Malignant Serous Effusions

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ABSTRACT

Background: *The investigation of serous effusions has an important place in cytopathology because it has diagnostic and prognostic value. The proper identification of the primary tumor with staging and grading has therapeutic and prognostic implications. In the current study, we evaluated the efficacy of two techniques for the diagnosis of malignant serous effusions.*

Methods: *Fresh samples of pleural, peritoneal, and pericardial fluids were evaluated for the present study. Ten milliliters of fluid were divided into two equal parts of 5 mL each, of which one was kept for conventional cytology and the other was used for the preparation of cell block.*

Results: *The evaluation of cell smear and cell block techniques showed predominantly moderate cellularity. Both in pleural and peritoneal fluids, cellularity was higher when using the cell block method as compared to the conventional smear method. Architectural patterns, including sheets, cell balls, papillae, glands, and three-dimensional clusters, were better appreciated in cell block than conventional smears. By using the cell block method, five additional cases were detected as malignant, which meant 6.66% more diagnostic yield for malignancy as compared to the cell smear technique.*

Conclusion: *This study revealed the importance of cell block preparation for supplementation of conventional smear technique. The same fluid extracted for the preparation of smear could be utilized to concurrently prepare cell blocks. The cell block technique provides additional diagnostic features and can help in further investigation by IHC studies for tumor staging and grading.*

Keywords: malignant serous effusions, cell block technique, cell smear technique, cytopathology.

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INTRODUCTION

Cytologic examination of body fluids obtained from the serous cavities is among the most common tasks performed in the practice of cytopathology (1). It is a relatively simple and non-invasive technique, which helps to conclude on the inflammatory, benign or malignant etiology of effusions (2). A positive diagnosis is always confirmatory; however, a negative result cannot rule out malignant causes (3). Cell block (CB) can be helpful in diagnosing malignancies, staging of lesions, and prognosis. The information regarding various non-infectious and infectious conditions, such as bacterial, viral fungal, and parasitic infections of the serous membrane, can also be assessed (1). Accurately, diagnosing cells as either malignant or reactive mesothelial cells in serous effusions is a common diagnostic problem. The lower sensitivity of cytodiagnosis of effusions is mainly attributable to overcrowding or overlapping of cells, cell loss, and changes due to different laboratory processing methods (1). The serous effusion is representative of a much larger surface area than that obtained by needle biopsy. The existence of reactive mesothelial cells, abundant inflammatory cells, and sometime paucity of representative cells are the issues in making the conclusive diagnosis in conventional smear (CS) preparations (4). The CB technique is another method of cytological diagnosis for serous effusions. It is one of the oldest methods for the evaluation of serous effusions (3). Due to cellular overlapping, delaying artifact, suboptimal processing, preparative cytotechnique, and leaving behind useful material causes lower diagnostic yield in the CS method. The residual material can be very useful in increasing the diagnostic yield by the CB method. The CB technique increases the sensitivity of detecting malignancies and can reduce false-positive interpretations (2). A recent method of CB preparation by using a 10% alcohol-formalin combination as fixative has shown to increase the cellularity and morphological details of cells (5). It is a simple, reproducible, and cost-effective method, which requires no extra material compared to other methods. The CB technique has many advantages over conventional cytology in improving the sensitivity of diagnosis, including preservation of tissue architecture like cell balls and papillae and three-dimensional clusters, excellent nuclear and cytoplasmic details and individual cell characteristics, and obtaining sections from the same material for special stains and immunohistochemistry (2). Hence, the present study emphasizes the role of the CB technique over CS technique in the cytodiagnosis of serous effusions. ❑

MATERIAL AND METHODS

This prospective study was carried on 100 consecutive patients undergoing paracentesis for effusion cytology by CS and CB method from November 2017 to October 2019. Fluid specimens were received in the cytology section, Department of Pathology, Kamineni Institute of Medical Sciences, Narketpally, Telangana, India.

Conventional smear technique

Fresh samples of pleural, peritoneal, and pericardial fluids were evaluated for the study. Fluids have been first examined with the naked eye. In the case of hemorrhagic fluid, 0.1% glacial acetic acid was added to haemolyze red blood cells. Ten milliliters of fluid were divided into two equal parts of 5 mL each, of which one was kept for conventional cytology and the other was utilized for the preparation of cell block. For conventional smear, 5 mL of fluid were centrifuged at 1000 rpm for five minutes and thin smears were prepared from the sediment. Smear was immediately fixed with 95% alcohol and stained with Haematoxylin-Eosin (H&E).

Cell block technique

The 5 mL of fluid for the CB technique was immediately fixed in 10% alcohol-formalin in the proportion of 1:1. After fixation, it was centrifuged at 1000 rpm for five minutes. After centrifugation, the supernatant was discarded and 3 mL of fresh 10% alcohol-formalin were added to the sediment and were kept for at least 24 hours. The next day, the sediment was scooped out on filter paper. The filter paper containing the sediment was routinely processed similarly to histopathological specimens. Paraffin-embedded 4-6 μ thick sections were routinely stained with H&E.

Interpretation of conventional smear and cell block

After studying all available clinical data, smears were categorized as benign, suspicious for malig-

nant and malignant lesions according to their morphology. We utilized the morphological criteria as described by Khan N *et al*. (6), which included cellularity, arrangement of acini, papillae, and cell balls, and cytoplasmic and nuclear details were determined in each case for classification of a cytomorphological pattern. Comparative evaluation of CS *versus* CB techniques was done and tabulation of cytomorphological characters was studied to identify the malignancy and most probable primary site. Statistical analysis was done for the present study. ❑

RESULTS

A total of 100 body cavity fluid samples (75 pleural fluids, 25 peritoneal fluids, and no pericardial fluids) were received and subjected to both smear and cell block techniques. Males had predominantly pleural and peritoneal effusion compared to females, as shown in Table 1. The subjects' mean age was 58.5±3.5, with the maximum numbers of samples being obtained from the age group of 51–60 years. Cirrhosis of the liver was the cause of peritoneal effusion in 11 (44%) out of 25 cases, followed by non-specific inflammation in eight (32%) cases, and renal conditions in six (24%) cases.

TABLE 1. Male and female distribution of body cavity fluids

Sl. No	Effusion	Male		Female	
		n	%	*n*	%
1	Pleural Effusion	53	73.61	22	78.57
2	Peritoneal Effusion	19	26.39	6	21.43
3	Pericardial Effusion	0	0.00	0	0.00
4	Total	72	100	28	100

TABLE 2. Cellularity in CS and CB of pleural and peritoneal fluids

Cellularity	Pleural Fluids				Peritoneal Fluids			
	CS		*CB*		*CS*		*CB*	
	n	%	*n*	%	*n*	%	*n*	%
Pauci	18	24.00	9	12.00	9	36.00	05	20.00
Moderate	35	46.66	41	54.66	11	44.00	14	56.00
Rich	22	29.34	25	33.34	5	20.00	06	24.00
Total	75	100.0	75	100.0	25	100.0	25	100.0

TABLE 3. Architectural of cells discovered in cell smear and cell block techniques

Architectural Pattern	Pleural fluid				Peritoneal fluid			
	CS		*CB*		*CS*			*CB*
	n	%	*n*	%	*n*	%	*n*	%
Singly/ scattered cells	44	58.67	15	20.00	5	20.00	4	16.00
Cell balls	5	06.66	9	12.00	3	12.00	3	12.00
Cell clusters	11	14.68	10	13.33	4	16.00	4	16.00
Papillae	4	05.33	6	08.00	0	00.00	0	00.00
Glands	6	08.00	16	21.33	5	20.00	8	32.00
Sheets	5	05.66	19	25.34	8	32.00	6	24.00
Total	75	100	75	100	25	100	25	100

Conventional smear and CB provided predominantly moderate cellularity. In both pleural and peritoneal fluids, cellularity was higher when using the CB method as compared to the CS method. Architectural patterns, including sheets, cell balls, papillae, glands, and three-dimensional clusters, were more accurately assessed by the CB technique than the CS one (Table 2).

Architectural pattern analysis of both pleural and peritoneal fluid samples showed that sheets, glandular pattern, cell clusters, and cell balls were more commonly observed in CB as compared to CS, whereas singly scattered cells were predominantly seen in conventional smear (Table 3).

Among 75 cases, a discrepancy between CS and CB was noted in five cases, out of which an analysis of pleural fluid samples showed that one case, previously reported as benign effusions by conventional smear, was identified as malignant effusion by the CB method. When using the CS technique, reported cases are diagnosed as benign due to singly scattered cells, with morphology being obscured by haemorrhagic background, plenty of inflammatory cells, necrotic material and reactive mesothelial cells. Other four cases have been previously reported as suspicious for malignancy in CS, but were diagnosed as malignant effusions in CB due to a clear cellular morphology studied by this method. The p-values were found to be significant in cases of suspicious for malignancy reports. So, by using the CB method, five additional cases were detected as malignant, meaning 6.66% more diagnostic yield for malignancy (Table 4).

In the peritoneal fluid, cytological diagnosis of malignant effusion was found in one case (04%), suspicion for malignancy in one case (04%), and the remaining 23 cases were diagnosed as benign effusions. By using the CB method, the suspicious case has been also detected to be malignant. Hence, 4% more diagnostic yield for malignant effusion was found by using the CB

CS & CB in pleural fluids					P values
Diagnosis by CS		Diagnosis by CB			
	n	*Benign*	*Suspicious*	*Malignant*	
Benign	66	65	00	01	0.21
Suspicious	04	00	00	04	0.02*
Malignant	05	00	00	05	0.91
Total	75	65	00	10	
CS & CB in peritoneal fluids					P values
Diagnosis by CS		Diagnosis by CB			
	n	*Benign*	*Suspicious*	*Malignant*	
Benign	23	22	00	00	0.00
Suspicious	01	00	01	01	0.92
Malignant	01	00	00	01	0.26
Total	25	22	01	02	

* Significant

TABLE 4. Analysis of discrepancies between CS and CB in pleural and peritoneal fluids

approach, although the p-values were not found to be significant, one of the reasons being the small size of the samples in the present study (Table 4).

Twelve body cavity fluid samples, including 10 pleural fluids and two peritoneal fluids, were diagnosed as malignant effusions by the CB method. Out of the 10 cases with malignant pleural effusions, the primary was known in all 10 cases, which included three cases from squamous cell carcinoma of the lung, five cases from adenocarcinoma of the lung, one case from carcinoma of the cervix, and another case from lymphoreticular neoplasm (NHL). An analysis of peritoneal effusion identified two cases of malignant peritoneal effusions, one case from primary lesion carcinoma colon, other cases from kidney, three (44.44%) cases of carcinoma lung in male subjects, and five (55.56%) cases lung carcinoma in females. The male to female ratio was 1:1 in malignant serous effusions. ❑

DISCUSSION

Of the total number of 100 body cavity fluids in the present study, 75% were pleural fluids and 25% peritoneal fluids. In serous effusions, various clinical conditions were noted, including pulmonary tuberculosis, pneumonia, renal diseases, cirrhosis, and non-specific inflammation. The most common etiological factor was pulmonary tuberculosis in pleural effusion and cirrhosis in peritoneal effusion. In pleural effusions, the primary neoplasms were noted from primary of carcinoma cervix in the lung of one case, and from non-Hodgkin lymphoma in other cases. Renal cell carcinoma and colon carcinoma were primary neoplasms in cases of peritoneal effusions. Cytological examination of serous effusions is important for diagnostic, therapeutic, and prognostic applications. Conventional smears have difficulties in diagnosis when reactive mesothelial cells are present, and also due to the paucity of representative cells, and abundance of inflammatory cells (7, 8). The detection of cancer cells in pleural or ascitic fluid is an indication of metastatic cancers, as tumors arising from mesothelial cells lining these spaces are rare. When present, tumor cells are usually numerous and they are frequently found in clusters; the glandular forms are more reliable on CB. The presence of mucin in tumor cells is an evidence that they have originated from glandular epithelium (9). In our technique, we used 10% alcohol-formalin as a fixative for CB preparation, which enabled us to obtain a better cellularity when compared to conventional smear, as formalin minimized the cell loss. It forms protein cross-links and a gel-like structure, which will not be dissolved by various chemicals used for processing the similar fixative used by Bodele *et al.* (7) Cell blocks may be prepared from residual tissue fluid and can be used in addition to smear for establishing a more definitive cytopathological diagnosis. In the past, reactive mesothelial cells have been responsible for the diagnosis of malignancy in CS due to the formation of rosettes, pseudoacini, or acini with/without the existence of nucleoli. The CB effectively puts both features in a proper perspective. The nucleoli in CB will not appear as prominent in CS; however, the pseduoacinar or acinar structures can be better appreciated in CB. Similar obser-

vations were noted in Dekker A *et al.* (10). More importantly, CB is a valuable tool in the evaluation of well-differentiated adenocarcinomas such as tumors of the breast, lung, or gastrointestinal tract. These tumors have few malignant characters in CS, while the presence of true acini, which is seen in CB, together with mucin, when stained for mucin, are indicative of malignancy. The other advantage of CB is represented by the fact that the concentration of cellular material in one small area that can be immediately evaluated with all cells lying in the same focal plane of the microscope. It bridges the gap between cytology and histology (10). The results of this study are comparable to other previous reports in the literature (11-14).

This study found that the yield of malignancy in the CB method was slightly greater than CS, which was consistent with findings from other studies (15-19). Ranjana *et al.* (13) found an increased yield for malignancy (0.66%) when using the CB method, which was comparable to the CS method, one of the reasons being a low percentage of cancer in both pleural and peritoneal effusions, as their study was performed in a general hospital serving mainly non-cancerous patients.

ES Kreethika Sri *et al.* (20) found a lower cellularity with cytosmears and an enhanced cellularity with cell block in hemorrhagic samples and suspicious cases of malignancy, which is in accordance with the results of the present study. The authors have also noticed that pattern analysis was only possible with CB to categorize primary *versus* metastatic samples.

F Guldaval *et al.* (21) found that CB provided a higher cellularity, architectural pattern and yield for malignancy as compared to the CS method; also, in 40% of cases, histological subtype was determined by CB especially in cases of adenocarcinoma.

Bodele *et al.* (7) showed that an additional 7% of malignant lesions were identified by the CB method. Dekkar A *et al.* (10) reported that combining the CB and CS methods for malignant lesions provided a double number of samples compared to CS technique only. By using the CB method, tumors were subsequently detected in 38% of patients who had a negative or atypical cytological report. Other studies have found a better diagnostic yield for malignancy when conventional smear was supplemented by CB preparations (12, 22). ❑

CONCLUSION

This study revealed the importance of cell block preparation for supplementation of conventional cell smear technique. The same fluid extracted for the preparation of cell smear could be utilized to concurrently prepare cell blocks. The cell block method provides additional diagnostic features and can help in further investigation by IHC studies for tumor staging and grading. ❑

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Disclaimer: All views in this paper express the author's position, and not the official position of the institution.

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