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ASCITIC FLUID CYTOLOGY IN SUSPECTED MALIGNANT EFFUSIONS WITH SPECIAL EMPHASIS ON CELL BLOCK PREPARATION

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ABSTRACT: BACKGROUND: Cytological examinations of serous effusions have been well accepted and a positive diagnosis often considered as definitive diagnosis. It helps for staging and prognosis of the patients for malignancy. Diagnostic problem arise to differentiate between reactive cells and malignant cells by conventional smear (CS) method. Cell block (CB) method provides better architecture, morphological features between reactive mesothelial cells and malignant cells and thereby increases the efficacy of cytodiagnosis. **AIMS AND OBJECTIVES:** To perform exfoliative cytology of ascitic fluid along with CB preparation and compare the diagnostic efficacy of CS methods verses CB method. **MATERIALS AND METHODS:** Total 48 samples were subjected to routine smear examination and cell block preparation. These samples are obtained from patients attending N.R.S. Medical College with clinical suspicion of malignancy. **RESULTS AND CONCLUSION:** Using a combination of the cell block and smear techniques yielded 24% more malignant cases in ascitic fluid. By cell block technique morphological and architectural pattern were better appreciated and increased sensitivity of cytodiagnosis.

KEYWORDS: Malignant effusion, cell block, smear, sensitivity.

INTRODUCTION: Cytological examination of serous fluid is one of the commonly performed and well accepted examinations. A positive diagnosis is often considered as definitive diagnosis. The first line of investigation of a suspected neoplastic lesion is often the cytological examination of fluid tapped from pleural, peritoneal and pericardial cavity. It is important not only in diagnosis but also staging and prognosis of the patients for malignancy.¹ Accurately diagnosing cells as being either malignant or benign reactive mesothelial cells in serous effusions is a common diagnostic problem in conventional cytological smears (CS). The lower sensitivity of cytodiagnosis of effusions is mainly attributable to bland morphological details of cells, overcrowding or overlapping of cells, cell loss, and changes due to different laboratory processing methods.² Cell block (CB) method provides better architecture, morphological features between reactive mesothelial cells and malignant cells and thereby increases the efficacy of cytodiagnosis.³ The present study was undertaken to assess the utility of cell-block preparation method in increasing the sensitivity towards cytodiagnosis of malignant effusions and compare the diagnostic efficacy of conventional cytological methods for effusion versus cell-block techniques.

MATERIALS AND METHODS: From February 2012 to July 2013, a total of 48 peritoneal effusion fluids were collected with clinical suspicion of malignancy in the Department of Pathology, NRS Medical College and Hospital, Kolkata after taking proper informed consent from the patients. Ten milliliters of fresh ascetic fluid sample was received and divided into two equal parts. One part subjected to conventional smear and staining and the remaining fluid was subjected to cellblock technique.

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Conventional Smear Technique: For conventional smear 5 ml of fluid was centrifuged at 2500 rpm for 15 minutes and a minimum of two thin smears were prepared from the sediment. One smear was air dried and stained with Leishman-Giemsa stain and the other smear was immediately fixed in 95% alcohol and stained with Papanicolaou stain.

Cell Block Technique: Remaining 5ml of fluid was processed for cellblock method. The fluid was centrifuged at 3000rpm for 5 minutes. The supernatant was decanted and the excess fluid was removed by inverting on the filter paper. To this sediment 2-3drops of plasma and 2-3 drops of thrombin was added and mixed well. Then this mixture of fluid was allowed to clot for 30 seconds. After that the clot was dislodged from the test tube and fixed in 1:1 mixture of alcohol formalin for one hour. Further the clot was transferred with the help of a pointed spatula on the top of lens paper inside the tissue cassette and then processed for paraffin embedding. Paraffin embedded 4-6 μ thick sections were routinely stained with Hematoxylin and Eosin stain. Special stain like Periodic Acid Schiff (PAS) was performed wherever necessary. After studying all the available clinical data and various investigation reports, based on morphology the smears were categorized as benign, suspicious for malignant and malignant lesions.

The following morphological criteria such as cellularity, arrangement of cells (Acini, papillae and cell balls), cytoplasmic and nuclear details were used for giving the cytological diagnosis. Comparative evaluation of conventional smear versus cellblock technique was done and tabulation of cytomorphological characters was studied to identify the malignancy and most probable primary site.

The cellular material in conventional smear is considered to be mild when there are 5-50 nucleated cells per high power field, moderate when there are 50-200 cells per high power field and marked when there are >200 cells per high power field. The cellular material in the cellblock is mild when there are 5-200 nucleated cells per high power field, moderate when 200-1000 cells per high power field and marked when there are >1000 cells per high power field.

RESULTS: Comparative evaluation of conventional smear versus cellblock technique was done. The data was tabulated and statistical analysis performed to see sensitivity, specificity, positive predictive value and negative predictive value to assess our study. Out of 48 ascitic fluids a mild male preponderance with male to female ratio 1.5:1 was noted. The maximum number of samples was in the age group of 51-60 years (25%). In males the maximum number of samples was in the age group of 51-60 years. In female the maximum number of samples was in the age group of 41-50 years and 51-60 years. Least number of samples was in the age group of 11-20 years. (Table 1). In case of ascitic fluids, in cellblock method 68% cases show marked cellularity while in conventional smear only 36% cases show marked cellularity. Singly scattered cells are more common in conventional smears and cell clusters, papillae are more common in cellblock.

Out of the 48 ascitic fluid samples cytological diagnosis of benign effusions was rendered in (76%) cases and suspicious for malignancy in (16%) cases whereas in cellblock malignant effusions were diagnosed in (32%) cases. There was no diagnosis of suspicious for malignancy in cellblock. (Table 1). In ascitic fluid, out of 48 cases difference in diagnosis were noted in 12 cases. Among them 4 cases were diagnosed as benign effusion on conventional smear. Other 8 cases were diagnosed as suspicious for malignancy. By cellblock method additional 12 malignant cases were diagnosed. There is 24% more diagnostic yield for malignancy. Total number of 17 ascitic fluid samples was diagnosed as malignant effusions by cell block method.

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Out of 17 cases of ascitic fluid primary was identified in 11 cases. The sensitivity and specificity of our study is 100% and 76% respectively. The positive predictive value is 41% and negative predictive value is 100%. Accuracy rate is 80%. Kappa value is 0.475 which indicates moderate agreement.

DISCUSSION: Cytological examination of serous fluids has increasingly gained acceptance in clinical practice to such an extent that a positive diagnosis often is considered the definitive test and obviates exploratory surgery.⁴ Reactive mesothelial cells, abundance of inflammatory cells and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional smears.⁵ In this study an attempt was made to prepare and analyze both smears and cellblock from the same specimen. Due consideration was given to age, sex, site of effusion and clinical and radiological findings to arrive at final diagnosis and also to identify primary malignant lesion. In the present study we evaluated conventional smears and cellblocks preparation for cellularity, architectural pattern, predominant cells, volume of obscuring background and the preservation of morphology. Here mild cellularity was observed in 40% samples and 24% samples show marked cellularity with conventional smear preparation where as in cell block method marked cellularity was observed in 52% samples. Chi-Square test for linear trend revealed statistically significant value indicating that the cellular yield was more with the cellblock method as compared to conventional smear method (Table 1).

The cellblock concentrated the cellular material into a small area which was useful in screening the material in lesser time. Similar findings were noted in studies by Thapar et al,² Dekker et al,⁴ Krogerus et al,⁶ Yang et al.⁷ Samples also show architectural patterns such as sheets, glands, papillae etc. In conventional smear 60% cases show single scattered cells whereas only 27% cases show single scattered cells in cellblock method. Here also Chi-Square test for linear trend revealed statistically significant value. Pseudoacinar or acinar structures and nucleoli were better appreciated in our study when compared to conventional smears. The reactive or atypical mesothelial cell which stimulate malignancy in smears were identified as reactive or mesothelial cells by cellblock method. Similar findings were noticed in Dekker and Bupp study.⁴

In a study by Dekker et al,⁴ the rate of recovery of tumor cells by cellblock preparation was double that obtained by smear alone. By using cellblock method tumours were subsequently demonstrated in 38% of the patient who had negative or atypical cytological reports. Thaper et al,² showed a diagnostic yield of 20% by cellblock preparations. The present study yielded 24% more malignant cases in ascitic fluid. In a study done by Khan et al,⁸ additional findings were diagnostic in 16% of malignant cases. Khan et al,⁸ in another study titled as "Usefulness of cellblock verses smears in malignant effusion cases" reported that the recovery rate for malignant lesions by cellblock preparation was 20% greater than that obtained for specimen examined in smear only.

In ascitic fluid, out of 48 cases difference in diagnosis was noted in 12 cases. Among them 4 cases were diagnosed as benign effusion on conventional smear. Other 8 cases were diagnosed as suspicious for malignancy. By cellblock method additional 12 malignant cases were diagnosed. There is 24% more diagnostic yield for malignancy. The degenerating mesothelial cells appear like signet ring cells, with large vacuoles replacing the nucleus to periphery and thus can be misleading. Similar findings were also observed in studies by Dekker et al.⁴ Takayagi et al,⁹ Chapman et al,¹⁰ and Vellios et al.¹¹ When conventional smears were compared with cellblock preparation for morphological preservation, the cellblock sections showed clearly recognizable cells with minimal shrinkage and

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aberrations. The cytomorphologic features were well maintained with minimal shrinkage and aberration. The findings were similar to the findings in the studies by Thaper et al,² Dekker et al.⁴ Nathan et al,³ and Takagi et al.⁹ Studies by Takagi et al,⁹ Vellios et al¹¹ and Sears et al¹² have suggested a clear preference for cellblock sections in cytological examination of effusions. Takagi et al⁹ concluded that the results of Papanicolaou method can be improved with increased volume of fluid to be centrifuged. Vellios et al¹¹ stated that Papanicolaou stain offers excellent nuclear and cytoplasmic details and errors may be avoided by proper attention to technical details. Out of 17 cases of ascitic fluid primary was identified in 11 cases which included 8 cases of ovarian tumor and 3 cases from carcinoma of GIT.

The advantages of the Cellblock Preparation are:

- Recognition of histologic patterns of diseases that sometimes cannot be identified reliably in smears.
- Possible processing of the multiple sections of the same material for routine staining, special staining and immunologic procedure,
- Less cellular dispersion, which permits easier microscopic observation than do the conventional smears,
- Less difficulty in spite of background showing excess blood on microscopic observation,
- Lower cost than the biopsies.
- Possibilities of storing slides for retrospective studies. Storage of the conventional smear is a practical problem.

The Disadvantages of the Cellblock Preparation are:

- Delay in diagnosis when compared to conventional smears.
- Loss of cellular materials and cytological details during processing.
- The techniques received not much attention, probably due to lack of standardized cost effective methods that achieve better diagnostic results.

The Limitations of the Present Study are:

- Inadequate sample in some cases resulting in inadequate cell block.
- Total no of cases are low, observation could have been more representative and more statistically significant if number of cases were more.
- Immunohistochemistry could not done, which could have been more informative.

CONCLUSION: Cytological examination of serous fluid is one of the commonly performed examinations. It is important not only in diagnosis but also staging and prognosis. The diagnostic performance of cytological study of fluid may be attributable to the fact that cell population present in sediment is representative of larger surface area than that obtained by needle biopsy. Cell block technique is simple, inexpensive and does not require any special training or instrument. Morphological features were better identified by cell block method when compared to conventional smear method. Multiple sections can be obtained if required for special stain or IHC study. It bridges the gap between cytology and histology. Therefore, a combined approach of conventional smears and cell block technique helps to get an additional diagnostic yield for malignancy in suspected pleural effusion and ascetic fluid samples.

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Sl. No.	Feature	CS Method No. %		CB Method No. %	
1	Benign	37	76	33	68
2	Suspicious for malignancy	08	16	00	00
3	Malignancy	03	08	15	32
	Total	48	100	48	100

Table 1: Ascitic fluid analysis

Chi Square test for linear trend in Ascitic fluid analysis in conventional smear and cell block =16.23, $P < 0.05$, $P = .0003$

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Fig. 1: Photomicrograph showing clusters and papillae of malignant cells in conventional smear (Leishman stain, 40x).

Fig. 2: Photomicrograph showing malignant cell clusters in cellblock (H & E, 40x).

Fig. 1

Fig. 2

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