



Cytological Study of Serous Effusions with Conventional Smear Cytology and Cell Block Technique

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ABSTRACT

Cell block technique when compared with conventional smear provides increased cellularity, preservation of architectural pattern with excellent morphology and clear background. Hence, we have conducted the study to compare cell block technique as compared with conventional cytological smear. An institution-based observation study was carried out on 100 serous fluids over one and half year. Serous fluid was divided into 2 parts. First part was used for conventional smear and second part was used for preparation of cell block using plasma thromboplastin method. Special stain and immunochemistry were done whenever required. Maximum number of patients belong to the age group of 41-50 years. Cellularity was more by cell block method as compared to conventional smear. 18 serous effusions diagnosed as malignant in cell block technique. Most common cause of malignant effusion was due to ovarian malignancy in 14 cases. Out of 100 cases discrepancy between conventional smear and cell block was observed in 14 cases. 3 cases which were reported as benign in conventional smear were diagnosed as malignant by cell block method. Out of 11 cases which were reported as suspicious as malignancy in conventional smear, 6 cases were diagnosed as malignant and 5 cases were diagnosed as benign by cell block method. Hence to reach conclusive diagnosis for cytological evaluation of effusion, cell block analysis is mandatory step in addition to conventional smear especially when conventional smear is suspicious for malignancy. Cell block technique can also help in identification of primary by immunohistochemistry.

INTRODUCTION

Cytological examination of body fluids has gained paramount importance to the diagnosis of malignancy and its primary site^[1]. It is important not only in diagnosis of malignant lesions but also helps in staging and prognosis^[2]. The most challenging aspect for a pathologist is to reliably distinguish between benign and malignant lesions in serous effusions^[3]. The accurate identification of cells as either reactive mesothelial cells or malignant cells is diagnostic problem in conventional cytologic smears. Conventional technique has lower sensitivity due to overcrowding of cells, loss of cellular architecture, increase in number of inflammatory cells and obscuring factors and less number of diagnostic cells contribute considerable difficulty in making conclusive diagnosis on conventional smears^[1,3,4]. The main advantages of cell block technique are it gives better cellular morphology, better nuclear and cytoplasmic preservation, intact cell membrane, crisp chromatin details, preservation of architectural pattern like papillae, acini, rosettes^[5]. Cell blocks are suitable for performing special stains and I.H.C. Hence the present study is undertaken to emphasize role of cell block technique over conventional smear in serous effusions and to study the feasibility of the use of immunohistochemistry in the diagnosis of malignancy of unknown origin.

MATERIALS AND METHODS

100 fresh samples of serous fluids (pleural, peritoneal and pericardial) were evaluated from January 2017 to June 2018. 15 ml of fluid was taken and divided into 2 parts, first 5 ml of fluid was used for conventional smear preparation and the second part of 10 ml was used for cell block preparation. For conventional smear 5 ml of fluid was centrifuged at 2500 rpm for 15 minutes and a minimum of three thin smears were prepared from the sediment. Smears are air-dried and stained with Leishman stain and other smear was immediately fixed in 95% alcohol and stained with Papanicolaou stain. Second part of fluid was processed by plasma thrombin method. The fluid was centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted and the excess fluid was removed by inverting on the filter paper. To this sediment 2-3 drops of plasma and 2-3 drops of thrombin was added and mixed by tapping and allowed to clot for 30 seconds. Then the clot was dislodged and fixed in formalin for 30 minutes. The clot was wrapped in filter paper and processed as a part of routine paraffin section histopathology. Hematoxylin and eosin staining was done. Special staining and immunohistochemistry were done whenever required. Data analysis was done

using SPSS statistical software version 20. Chi square test was used for data variables. The p value of <0.05 is considered as significant.

RESULTS AND DISCUSSIONS

100 cases of serous effusions were subjected to CS and CB method technique. Out of 100 serous effusions, 52 cases were of pleural fluid, 46 cases were of ascitic fluid and 2 cases of pericardial fluid. Maximum number of samples were in the age group of 41-50 years. Least number of samples were in the age group of less than 20 years. Males predominantly have pleural effusion and females predominantly had ascitic effusion. The cellularity in conventional smear showed minimal cellularity in 11 cases (11%), moderate cellularity in 71 cases (71%) and marked cellularity in 18 cases (18%). Cellularity in cell block were minimal in 8 cases (8%), moderate in 47 cases (47%) and marked in 45 cases (45%). Cellularity was more by cell block method as compared to conventional smear. (Table no. 1).

Table 1: Comparison of Cellularity of CS and CB in Serous Effusion

Cellularity	Conventional smear	Cell Block	Inference
Minimal	11	8	P value < 0.001
Moderate	71	47	
Marked	18	45	

P value <0.5 is significant.

Architectural patterns such as glands, sheets, three dimensional clusters and cell blocks most commonly observed in cell block method with P value of 0.001. (Figure 1 and 2).

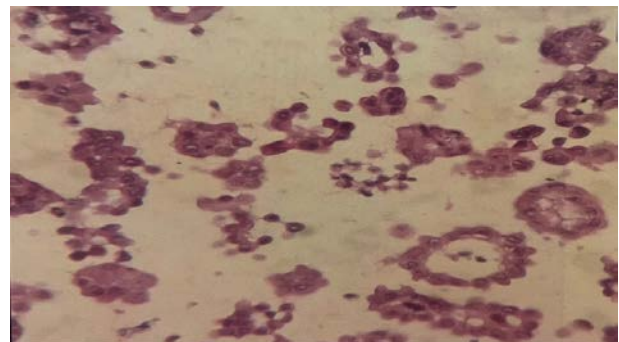


Fig. 1: Malignant Cells in Clusters and Acini in Cell Block

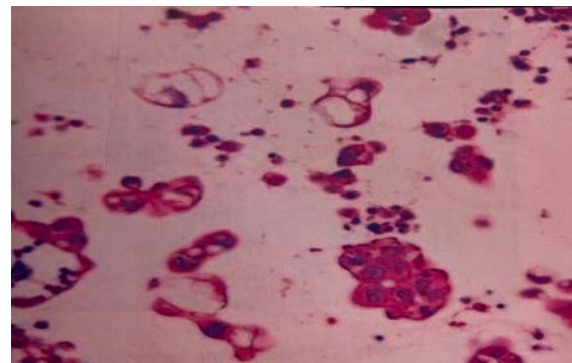


Fig. 2: Cluster of Malignant Cells and Signet Ring Cells in Cell Block

After analysis of samples, they were categorized as benign, suspicious for malignancy and malignant. By cell block method additional 9 cases were detected as malignant. Out of 100 cases discrepancy between CS and CB method was observed in 14 cases. Analysis of these 14 cases of serous effusions showed that 3 cases which were reported as benign effusions in conventional smear were diagnosed as malignant by cell block method. Out of 11 cases which were reported as suspicious for malignancy in conventional smear, 6 cases were diagnosed as malignant and 5 cases were diagnosed as benign by cell block method. (Table no 2).

Table No.2: Analysis of Discrepancies Observed Between CS and CB
Conventional smear Cell Block

Benign	Suspicious	Malignant	Benign	Suspicious	Malignant
77	0	0	77	0	0
3	0	0	0	0	3
0	6	0	0	0	6
0	5	0	5	0	0
0	0	9	0	0	9
Total=80	11	9	82	0	18

18 serous effusions were diagnosed as malignant effusion by cell block method in the present study. Out of 18 malignant effusions, we identified primary site in 15 fluids which was confirmed on histopathology. Out of 15 cases, 14 cases of ovarian malignancy in ascitic fluid and one case of cholangiocarcinoma in pleural fluid. In the remaining 3 cases, identification of primary site was done with the help of clinical history, radiological investigation and immunomarkers. One case showed diffuse positivity for LCA, focal positivity for bcl2, weak positive for CD20 and negative for CD3 in a known case of Non-Hodgkin lymphoma. After applying all these panel of immunomarkers case was diagnosed as B cell NHL. The remaining 2 cases of malignant ascitic fluid which were from female, we identified primary site with the help of radiological investigations and after applying immunomarkers CK7 and CK20. They showed diffuse CK7 positivity and CK20 negativity. So primary site was identified as carcinoma of ovary.

The cytological examination of serous effusions has increasingly gained acceptance into an extent positive diagnosis is often considered as definitive test and obviates exploratory surgery^[6]. Distinction between benign reactive mesothelial cells from malignant cells is critical in cytological diagnosis of body fluids. The overlapping morphological features between these two types of cells pose a major diagnostic challenge in routine cytological practice^[7]. Though the preparation procedure for conventional smear is much simpler than CB method but it has limitations like lack of tissue architecture. Appreciation of architecture in CB makes diagnosis easier^[8]. In the present study, plasma

thromboplastin method was used for cell block preparation. In this method concentrates are more cellular material that forms more solid button because of formation of clot, better cellular preservation and the disadvantage is background staining on IHC. Kulkarni MB et al and Rekhi B et al used similar method for cell block preparation^[9,10]. Thaper *et al* used sediment method with 10% alcohol formalin as a fixative^[3]. In the present study of total of 100 cases of serous effusions, the majority 52(52%) cases were of pleural effusion followed by 46(46%) ascitic fluid whereas the least number 2(2%) of cases were from pericardial effusion. M Bhanvadia Viral *et al* found that out of 150 fluid samples 79 (52.5%) were pleural, 69 (46%) were ascitic fluid and 2 (1.5%) were pericardial effusion^[11]. In our study, the distribution of serous effusions was similar to M Bhanvadia Viral *et al*. Age of patient in our study who had serous effusion ranged from < 20 years to >60 years with maximum number of cases in 5th and 6th decade in both male as well as female. This was similar to the study done by Gude A et al and Bindu^[12,13]. In the present study of 100 cases CB method showed mild cellularity in 8 cases (8%), moderate cellularity in 47(47%) cases and marked cellularity in 45(45%) cases. Castro Villebon D *et al* found cellularity in cell block was seen in 29.6% of cases which is lower than that of present study^[14]. In our study the p value of cellularity between CS and CB is <0.001. The p value for retention of architecture between CS and CB is 0.001. Therefore statistically there is highly significant difference between CS and CB. Similar finding was observed by Shivkumarswamy^[15]. When conventional smears were compared with cell block preparation for morphological preservation cell block sections showed clearly recognizable cells with minimal shrinkage. The finding were similar to the finding in the studies done by Nathan et al and Thaper^[16,3]. In the present study of 100 cases most of the cases were of benign category with 80% on CS while 82% on cell block. In suspicious for malignancy category 11 cases were reported as suspicious for malignancy on CS and none on CB. Out of 11 cases were reported as suspicious for malignancy in CS, 6 cases were diagnosed as malignant and 5 cases were benign in cell block. The cell block yields higher diagnosis of malignancy which was missed by conventional smears. The p value is <0.001 which shows statically significant difference between two methods. In our study amongst malignant serous effusion diagnosed by cell block, the ovarian carcinoma was commonest accounting for 16 cases (78.5%) followed by cholangiocarcinoma 1 case (7.14%) and NHL 1 case (7.14%). Most of the malignant neoplasms in peritoneal fluid in the present study was from carcinoma of ovary which is similar to study done by

Monte^[17]. Malignancy was diagnosed in 9% by CS and 18% by CB in the present study. Thus cell block has increased diagnostic yield of malignancy by 9%. Study done by Flint et al found increase in malignancy yield was also 9% and study done by Bodele *et al* was 7%^[2,18]. The p value is <0.001 which shows a statically significant difference between these two methods.

CONCLUSION

Cell block technique is simple and reproducible and uses routine laboratory reagents and processing. Use of cell block technique eliminated the suspicious for malignancy category giving more definitive diagnosis and showed additional increase in diagnostic yield of malignancy.

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