Original Article

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Pathology Section

The Role of the Cell Block Method in the Diagnosis of Malignant Ascitic Fluid Effusions

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ABSTRACT

Background: The Cell Block (CB) technique is one of the oldest methods which is used for the evaluation of body cavity fluids. The accurate identification of the cells as either malignant or reactive mesothelial cells is a diagnostic problem in cytological conventional Smears (CS). As compared to the older methods, a new method of cell block preparation which is being used, which uses 10% alcohol-formalin as a fixative, increases the cellularity, gives better morphological details and helps in improving the sensitivity of the diagnosis. Multiple sections can be obtained by the CB method for the special stains and immunohistochemistry studies.

Aims: To know the role, utility and the sensitivity of the cell block method in the diagnosis of malignant ascitic fluid effusions.

Materials and Methods: This study was conducted in the Cytology Section of the Department of Pathology. 44 peritoneal fluid samples were subjected to a diagnostic evaluation for over a period of 20 months. The cell blocks were prepared by using 10% alcohol-formalin as a fixing agent along with the CS. The cellularity, architectural patterns, morphological details and the cytoplasmic and the nuclear details were studied both in the CS and the CB methods. Mc. Naemer's χ^2 test was used to identify the additional yield for malignancy which was obtained by the CB method.

Results: The additional yield for malignancy was 13.63% more as was obtained by the CB method.

Conclusions: The CB method provides high cellularity, better architectural patterns, morphological details and an additional yield for malignant cells. Therefore, the CB technique could be considered as a useful adjuvant in evaluating the fluid cytology for a final cytodiagnosis, along with the routine CS method.

Key Words: Cell block, Conventional smear, Ascitic fluid, Cytodiagnosis

INTRODUCTION

The cytological examination of serous fluids is important in the diagnosis, staging and the prognosis of malignant lesions. The cytodiagnosis which is made by conventional smears has got a lower sensitivity due to the overcrowding of the cells, cell loss and also due to the different laboratory processing methods. The accurate identification of the malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears [1].

The cell block technique is one of the oldest methods which is used for the evaluation of the body cavity fluids [2]. The routine use of CB by agar or plasma thrombin is not cost effective, as it needs additional material. A new method of the CB preparation which uses 10% alcohol-formalin as a fixative, which is being used, is a simple, inexpensive method, and it does not require any special training or instrument. This method increases the cellularity, gives better morphological details and it also improves the sensitivity of the diagnosis [1]. Therefore, the CB technique can be considered as a useful adjuvant in evaluating the fluid cytology for a final cytodiagnosis, along with the routine CS method.

MATERIALS AND METHODS

Peritoneal fluids were collected for cytological evaluation in the Cytology Section for a period of 20 months. Ten milliliters of fresh peritoneal fluid sample was divided into two equal parts of five milliliters each. One part was subjected to the conventional smear cytology technique and the other part for the cell block technique. Thus, the same sample was evaluated for a comparative study.

The Conventional Smear Technique

The 5 milliliter sample was centrifuged at 2500 rpm for 15 minutes. A minimum of 2 thin smears were prepared from the sediment. One smear was prepared after air drying and it was stained with the May-Grünwald-Giemsa stain. The other smear was immediately fixed in 95% alcohol and it was stained with the Papanicolaou stain.

The Cell Block Technique

The remaining 5ml sample was subjected to fixation for one hour by mixing it with 5ml of 10% alcohol–formalin (i.e., 9 parts of 90% alcohol and one part of 7.5% formalin). This 10 ml fluid was centrifuged at 2500 rpm for 15 minutes after one hour. A further 3ml of fresh 10% alcohol–formalin was once again added to the sediment after discarding the supernatant and it was kept for 24 hours. On the next day, the sediment which contained the cell button of the peritoneal fluid sample was scooped out on to a filter paper. This cell button was processed along with other routine biopsy specimens. After paraffin embedding 4–6 µ thickness sections were prepared from this cell button [Table/Fig-1] (Cell block), and they were stained with the hematoxylin and eosin stain. Special stains like the Periodic Acid Schiff (PAS) and Mucicarmine were performed wherever they were necessary.

The Interpretation of CS versus CB

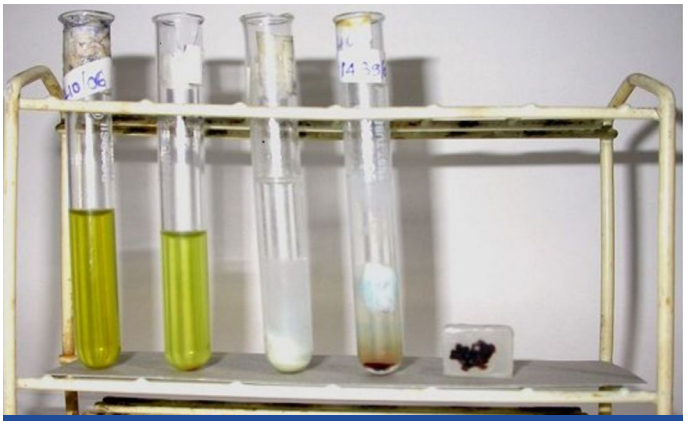
The samples were studied in detail, taking into account the available clinical data, various investigation reports and microscopic

details. The samples were categorized as benign, suspicious for malignancy, or malignant lesions. The morphological criteria that were taken into account, included the cellularity, the arrangement of the cells (acini, papillae and cell balls) and the cytoplasmic and the nuclear details. All these criteria were put together and they were used for the categorization of the sample. The cytomorphological characters were studied in detail to identify the malignancy and the most probable primary site. A comparative evaluation of the CS versus the CB techniques was conducted.

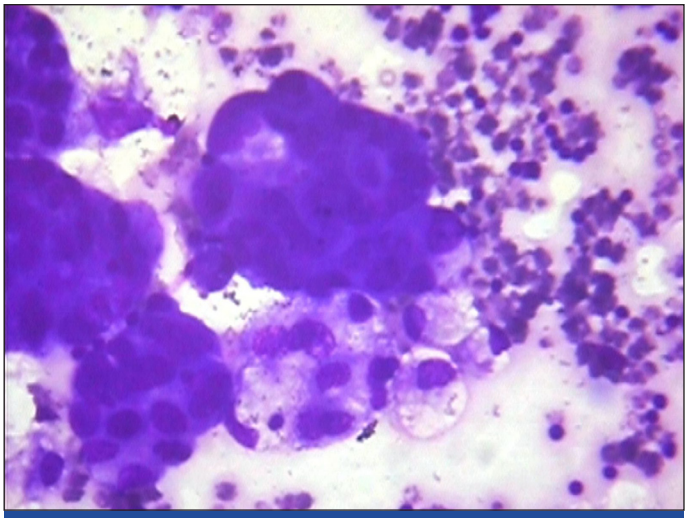
RESULTS

44 peritoneal fluid samples were subjected to the CS and the CB techniques. The ages of the patients ranged from 21 to 80 years. The maximum number of samples were from the 51–60 years age group. The female patient's samples (23) outnumbered the male patient's samples. The cellular yield which was obtained by the CB method was more when it was compared to that which was obtained by the CS method. Architectural patterns such as, glands, three-dimensional cell clusters, cell balls and sheets, were commonly observed in the CB method as compared to the singly scattered cells, glands and cell clusters which were found in the CS findings. After the analysis of the above samples, they were categorized as benign, suspicious for malignancy, [Table/Fig-2] or malignant samples [Table/Fig-3]. By the CB method, an additional 6 cases were detected as malignant, that is, a 14% more diagnostic yield for malignancy. These samples were reported as either suspicious for malignancy or benign samples. Further analysis showed a discrepancy in 08 cases [Table/Fig-4]. In the CS method, out of 4 reported benign samples, one case was reported as florid mesothelial hyperplasia, and the other 3 samples were misdiagnosed, as the morphology was obscured by a haemorrhagic background, plenty of inflammatory cells and reactive mesothelial cells. However, these four samples were reported as malignant by the CB method. Out of the 4 samples that were reported as suspicious for malignancy by the CS method, 2 samples were diagnosed as malignant effusions and the other 2 as benign lesions by the CB method.

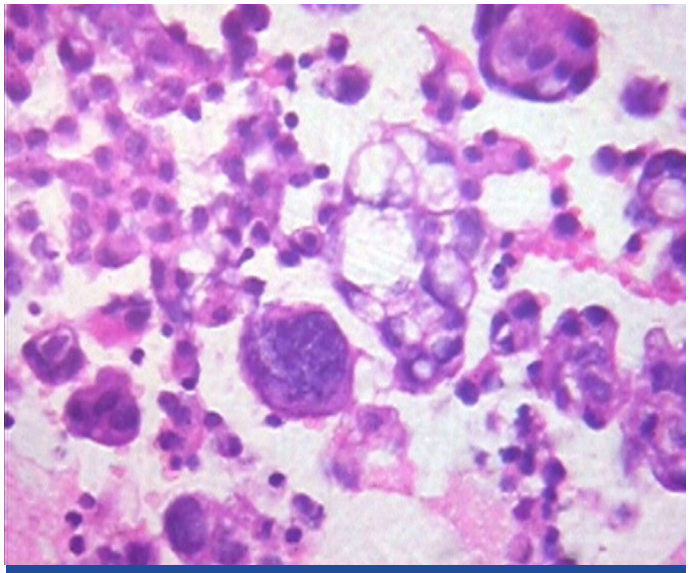
The malignant effusions were more common in females than in males. The female-to-male ratio was 2:1 for the malignant effusions. The most common primary malignancy, identified was from the ovary. Out of 13 cases of malignant peritoneal effusions, the primary was known in nine cases, which included 5 cases of carcinoma of the ovary and one case each of carcinoma of the colon, liver, cervix, and the urinary bladder. In the remaining 4 cases, the primary malignancy could not be detected, as the patients were lost to follow-up. The statistical analysis of these 44 samples showed a high cellular yield by the CB method than by the CS method. Mc. Naemer's χ^2 test was used for analyzing the benign and the malignant lesions by the CB and the CS methods in which the *P* value was found to be highly significant. The results showed 100% sensitivity by the CB method in the diagnosis of malignancy. Therefore, in this study, the utility of the CB method in the cytodiagnosis of malignant effusions was found to be highly significant as compared to the CS method.



[Table/Fig-1]: Photograph showing peritoneal fluid samples for CS, CB, and Paraffin block



[Table/Fig-2]: Photomicrograph showing cell ball of malignant cells in CS (Giemsa 40X)



[Table/Fig-3]: Photomicrograph showing cell ball of malignant cells, pleomorphic cells in CB (H&E 40X)

Sl. No.	Feature	CS method		CB method	
		No	%	No.	%
1	Benign	33	75	31	70
2	Suspicious	04	09	00	00
3	Malignancy	07	16	13	30
	Total	44	100%	44	100%

[Table/Fig-1]: Analysis of discrepancies between CS and CB in the peritoneal fluid

DISCUSSION

The cytological examination of serous effusions is of paramount importance in diagnostic, therapeutic and prognostic implications. It is important not only in the diagnosis of malignant lesions, but it also helps in the staging and the prognosis of these lesions [3].

The malignant cells in the pleural or the ascitic fluids were almost always indicative of metastatic tumours, as primary malignancies which arose from the mesothelial cell lining were rare. When a primary malignancy was present, the tumour cells were usually found to be numerous and they were seen in clusters. A positive effusion for malignant cells is an important prognostic indicator in oncologic patients. The development of a malignant pleural effusion is a common complication of cancers like pulmonary and gastric carcinomas [4]. Malignant neoplasms, especially lymphoid neoplasms, represent a major cause of death in children and in these cases, a cytological examination is very useful for their management [5]. Hence, presently, the examination of body fluids for the presence of malignant cells has been accepted as a routine laboratory procedure, not only for the detection of unsuspected cancers, but also for the detection of metastasis of an unknown primary origin [1,3,5].

Beale introduced the paraffin-block method for serous effusions in 1895 [6]. In 1896, Bahrenberg first described the cell block technique and it was commonly used after Mandlebaum reported the finding of actinomyces in a cell block [7].

In the CS method, reactive mesothelial cells, an abundance of inflammatory cells and a paucity of representative cells contribute to the considerable difficulties which are faced in making conclusive diagnosis. The reactive mesothelial cells which are common in hepatic cirrhosis, allergic pleurisy, polyarteritis, pulmonary infarcts and in long standing effusions, of cardiovascular diseases, may show reactive changes such as cytomegaly, nucleomegly, multi-nucleation, mitotic figures and a high N/C ratio. Another limitation of the conventional cytological examination of effusions is that it has a sensitivity of only 40–70% for detecting the presence of malignant diseases, due to the overcrowding of the cells, cell loss and also due to the different laboratory processing methods [8]. The difficulty is either secondary to the marked atypia of the mesothelial cells which is caused by the microbiological, chemical, physical, immunological, or the metabolic insults to the serous membranes or due to the subtle cytomorphological features of some malignant neoplasms [9]. The problem may become compounded due to the artifacts which are caused by poor fixation, preparation, or staining techniques [8,9]. For this reason, in this study, an attempt was made to prepare and to analyze both the CS and the CB which were prepared by using 10% alcohol- formalin as a fixative, from the same specimen.

Although the preparation of CS is a much simpler procedure than that of paraffin sections, it has limitations, that is, a lack of the tissue architecture. In some cases, the appreciation of the tissue architecture could make the diagnosis easier [10]. The storage of the CS slides is also a practical problem [10,11].

The CBs which are prepared from the residual tissue and fluids can be particularly useful for the identification of the tumours that cause diagnostic difficulties on smears. This technique is simple, reproducible and safe. Further, the effectiveness of the cellblock lies in the availability of the diagnostic material for the further histological examination, histochemistry and IHC studies for a better classification of the tumour and for the identification of infectious causes by using microbiologic stains [3,6,9,10].

In this study, the paraffin block gave a concentrated material in smaller fields, a more frequent appearance of the organoid pattern and cells in the same focal plane.[10,11,12] The serial sections which were made from even a minute amount of cellular material from various types of the sample showed a high cellularity with an excellent morphologic preservation [13]. The diagnosis of carcinoma which is more reliable when it is based upon the cell clusters rather than on the individual cells [7,14]. The paraffin block effectively puts the morphological features in their proper perspective, i.e., the presence of the nucleoli and the pseudoacinar or the acinar structures. It is a valuable tool which can be used for the identification of the acinar structures in a majority of adenocarcinomas and the papillary nature in some cases. The glandular forms can be more reliably diagnosed on CBs. The demonstration of mucin in the tumour cells is an evidence that they originate from a glandular epithelium [2,11,13]. More important still, this CB is a valuable tool which can be used for the evaluation of well-differentiated adenocarcinomas such as tumours of the breast, lung, or the gastrointestinal tract. These tumours have few malignant characters in CS, while the presence of the true acini is seen in the CB, together with mucin, when it is stained for mucin, and these are are indicative of a malignancy [2,7,14].

The main advantages of the CB procedure include: recognition of the histological patterns of diseases, the possibility of studying multiple sections by routine staining, special staining and by IHC studies, lesser cellular dispersal, less difficulty on microscopic observation and the possibility of storing the slides for retrospective studies [1,3,6,11,13].

The disadvantage with the cellblock technique is a delay in the diagnosis when it is compared to the conventional smears and sometimes, the risk of losing material during the processing [14]. Some mesothelial cells, because of centrifugation artefacts, may form rosettes or pseudoacini which can be the sources of a misdiagnosis [15].

The CBs from serous effusions can be prepared by various methods. They can be prepared by adding a few drops of old plasma and thrombin solution to the centrifuged button and by fixing it in 95% alcohol and 5% formalin. Fixatives such as 2% agar with 10% formalin can also be used for the cellblock preparation [15]. These techniques have received not much attention, probably due to the lack of standardized cost effective methods that can achieve better diagnostic results. The routine use of cell block by the agar or the plasma thrombin methods is not cost effective, as it requires additional materials and the consumption of extra time as compared to the earlier conventional methods [16].

The CB technique which uses 10% alcohol–formalin as a fixative, was found to be simple and inexpensive and it did not require special training or special instruments. By using formalin, the proteins would become cross linked and a gel would be formed, which could not be dissolved in any material during sample processing, thus minimizing the cell loss [3]. To achieve the maximum usefulness of CB, the fixation and the processing of the samples had to be modified. By using 5-10% formalin, results which were comparable to those of the biopsy reports were obtained [16].The use of an alcohol based fixative provides a better preservation of the antigenicity and also cytomorphological features which are comparable to those of the conventional smears [10].

Histochemical staining methods can easily be performed on the sections which are prepared from CB. For the histochemical studies, various special stains such as PAS, PAS-Diastase, Ziehl-Neelsen and Gomori-Methenamine Silvernitrate can be done [10]. The CB technique is a valuable method, particularly when the IHC staining is required for a battery of markers. The IHC staining,

when it is applied to the cellblock preparations, provides the same accuracy as do the histological specimens [3,6,11].

By using a combination of the CS and the CB methods for the reporting of malignant effusions, the primary site could be determined with 81% accuracy [14,17]. On correlating the clinical, radiological and the cytological features, the primary site could be determined with 90% accuracy [17].

In this study, the additional yield for the malignancy was found to be 14% more by CB as compared to that which was obtained by the CS findings. Our results correlated with those of a study which was done by Khan et al. [14,17] According to various studies, an additional diagnostic yield for malignancy was noted if the conventional smear technique was supplemented by the cellblock method [1,6].

Among the peritoneal effusions in our study, ovarian carcinoma (69%) was the commonest primary, followed by one case each of carcinoma of the GIT, liver, cervix and the urinary bladder (8%). Bonito et al., [18] study, reported a similar pattern of primary lesions. The CB study provided additional information for a definitive diagnosis, as it allowed the recovery of even minute cellular materials and it was valuable for the histochemical and the immunohistochemical methods [2,13].

To conclude, the present study results showed that the CB technique which used 10% alcohol–formalin as a fixative, was a simple, safe, reproducible and inexpensive method, which did not require any special training or instrument. This method yielded more cellularity with better architectural patterns and it improved the cytodiagnosis of additional malignancies by 14%. Hence, the CB technique can be recommended as a useful adjuvant in evaluating the fluid cytology for a final cytodiagnosis, along with the routine CS method.

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