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Original Article

Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology

ABSTRACT

Background: The cytological examinations of serous effusions have been well-accepted, and a positive diagnosis is often considered as a definitive diagnosis. It helps in staging, prognosis and management of the patients in malignancies and also gives information about various inflammatory and non-inflammatory lesions. Diagnostic problems arise in everyday practice to differentiate reactive atypical mesothelial cells and malignant cells by the routine conventional smear (CS) method.

Aims: To compare the morphological features of the CS method with those of the cell block (CB) method and also to assess the utility and sensitivity of the CB method in the cytodiagnosis of pleural effusions.

Materials and Methods: The study was conducted in the cytology section of the Department of Pathology. Sixty pleural fluid samples were subjected to diagnostic evaluation for over a period of 20 months. Along with the conventional smears, cell blocks were prepared by using 10% alcohol–formalin as a fixative agent. Statistical analysis with the 'z test' was performed to identify the cellularity, using the CS and CB methods. Mc. Naemer's χ^2 test was used to identify the additional yield for malignancy by the CB method.

Results: Cellularity and additional yield for malignancy was 15% more by the CB method.

Conclusions: The CB method provides high cellularity, better architectural patterns, morphological features and an additional yield of malignant cells, and thereby, increases the sensitivity of the cytodiagnosis when compared with the CS method.

Key words: Cell block; conventional smear; pleural effusions; sensitivity.

Introduction

Cytological examination of serous fluids is one of the commonly performed investigation. The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. The cell block (CB) technique is one of the oldest methods for the evaluation of body cavity fluids.[1] However, a new method of cell block preparation by using 10% alcohol-formalin as a fixative was used, to identify the sensitivity of the diagnosis in comparison with the conventional smear (CS) study. The main advantages of the CB technique are preservation of tissue architecture and obtaining multiple sections for special stains and immunohistochemistry.[2]

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Materials and Methods

Pleural fluids were collected for cytological evaluation in the cytology section of the Department of Pathology, from September 2005 to April 2007. Ten milliliters of fresh pleural fluid sample was received. It was divided into two equal parts of five milliliters each. One part was subjected to conventional smear cytology and the other part for the cell block technique. Thus, the same sample was evaluated for a comparative study.

Conventional smear technique

The five milliliter sample was centrifuged at 2500 rpm for 15 minutes. A minimum of two thin smears were prepared

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from the sediment. One smear was prepared after air drying and stained with the May-Grünwald-Giemsa stain. The other smear was immediately fixed in 95% alcohol and stained with the Papanicolaou stain.

Cell block technique

The 5 mL sample that remained was subjected to fixation for one hour by mixing with 5 mL of 10% alcohol–formalin (i.e., nine parts of 90% alcohol and one part of 7.5% formalin). After one hour, this 10 ml fluid was centrifuged at 2500 rpm for 15 minutes. The supernatant was discarded and a further 3 mL of fresh 10% alcohol–formalin was once again added to the sediment and it was kept for one day. On the following day, the sediment containing the cell button of the pleural fluid sample was scooped out on to the filter paper and this cell button sediment sample was processed along with other routine histopathological specimens. The paraffin embedded cell button (cell block) sections of 4–6 μ thickness were prepared and stained with the hematoxylin and eosin stain. Special stains like the periodic acid Schiff (PAS) and mucicarmine were performed wherever necessary.

Interpretation of CS versus CB

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

Results

Sixty pleural fluid samples were subjected to the CS and CB method techniques. The age ranged from 18 to 90 years. Maximum samples were from the 51–60 year age group (21%). The least number of samples was from the age group of 11–20 years (2%). Male patient samples (thirty-five) outnumbered the female patient samples. Cellularity was more by the CB method when compared to the CS method. Architectural patterns, such as, glands, sheets, three-dimensional cell clusters and cell balls were commonly observed in the CB method, whereas, singly scattered cells were predominant findings in CS.

After analysis of the above samples, they were categorized as benign, suspicious for malignancy, [Figure 1] or malignant samples [Figure 2]. By the cell block method, an additional nine cases were detected as malignant, that is, 15% more diagnostic yield for malignancy. These samples were reported as either suspicious for malignancy or benign samples. Further analysis showed a discrepancy in 12 cases [Table 1]. In the conventional smears, out of seven reported benign samples, two were reported as florid mesothelial hyperplasia,

Table 1: Analysis of discrepancies between CS and CB in the pleural fluid

Conventional smear method			Cell block method		
Benign	Suspicious	Malignant	Benign	Suspicious	Malignant
7	0	0	0	0	7
0	2	0	0	0	2
0	3	0	3	0	0
0	0	1	0	0	1
47	0	0	47	0	0
Total=54	5	1	50	0	10

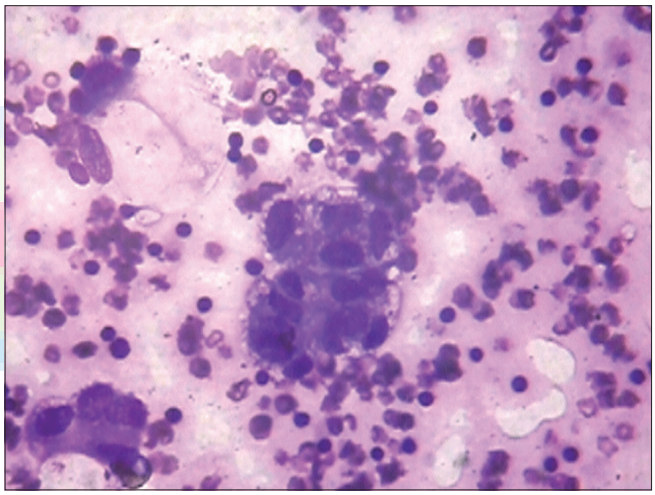

Figure 1: Photomicrograph showing scattered and clusters of suspicious cells in CS (Giemsa, ×400)

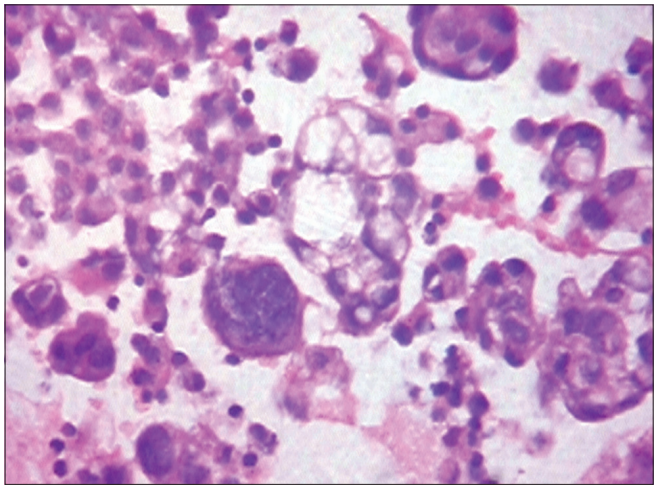

Figure 2: Photomicrograph showing bizarre shaped cells with pleomorphic nuclei in cell block from the same sample shown in Figure 1 (H and E, ×400)

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four samples were misdiagnosed, as the morphology was obscured by a hemorrhagic, dirty background, plenty of inflammatory cells and reactive mesothelial cells. One more sample was misdiagnosed as an inflammatory smear. However, these seven samples were reported as malignant by the CB method. Out of the five samples that were reported as suspicious for malignancy in CS, two samples were diagnosed as malignant effusions and the other three as benign lesions by the CB method.

The malignant effusions were more common in females than males. The female-to-male ratio was 3:1 for malignant effusions. The most common primary identified was from the breast. Out of 10 cases of malignant pleural effusions, the primary was known in seven cases, which included three cases of carcinoma breast from female patients and two cases each of carcinoma of the lung (one from a male and another from a female patient) and gastrointestinal tract (one from a male and another from a female patient). In the remaining three cases, the primary could not be detected as the patients were lost to follow-up.

Statistical analysis of these 60 samples showed high cellular yield by the CB method rather than the CS method. For this a *z* test was done that showed a *P* value of 0.038. Mc. Naemer's χ^2 test was used for analysing benign and malignant lesions by the CB method and CS methods in which the *P* value was 0.0021. Results showed 100% sensitivity by the CB method in the diagnosis of malignancy. Therefore, in this study, utility of the CB method in the cytodiagnosis of malignant effusion was highly significant as compared to the conventional smear method.

Discussion

The cytological examination of serous effusions has increasingly gained acceptance in clinical medicine, to such an extent that a positive diagnosis is often considered the definitive test and obviates explorative surgery. It is important not only in the diagnosis of malignant lesions, but also helps in staging and prognosis.[3]

The development of malignant pleural effusion is a common complication of cancers like pulmonary and gastric carcinomas.[4] Examination of fluids from the serous cavities of the body is an essential component of management in adult patients. Malignant neoplasms, especially lymphoid neoplasms, represent a major cause of death in children and in these cases cytological examination is very useful in their management.[5]

One of the most common problems in CS cytology is to distinguish reactive mesothelial cells from metastatic neoplasms. The difficulty is either secondary to marked atypia of mesothelial cells caused by the microbiological, chemical, physical, immunological, or metabolic insults to the serous membranes or to the subtle cytomorphological features of some malignant neoplasms, particularly well-differentiated adenocarcinomas. The problem may become compounded by artefacts from poor fixation, preparation, or staining techniques.[6] Although the preparation of CS is a much simpler procedure than that of paraffin sections, it has limitations, that is, lack of tissue architecture. In some cases, appreciation of tissue architecture make diagnosis easier.[7] Another limitation of the conventional cytological examination of effusions is that it has a sensitivity of only 40–70% for the presence of malignant disease due to overcrowding of cells, cell loss and different laboratory processing methods. Others like reactive mesothelial cells, abundance of inflammatory cells and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional smears.[8]

Since the introduction of the CB technique by Bahrenburg nearly a century ago, it has been used routinely for processing fluids. In 1928, Zemansky concluded that the CB method was superior to the CS technique and that examination of materials other than pleural and ascitic fluids was unreliable. Cancer cells in the pleural or ascitic fluid are almost always indicative of metastatic cancer, as tumors arising from mesothelial cells lining these spaces are rare. When present, the tumor cells are usually numerous and frequently clusters may be found. The glandular forms are more reliable on CB. The demonstration of mucin in the tumor cells is evidence that they originate from a glandular epithelium.[9] Diagnostic problems arise whenever there is only marginal morphological distinction, for example, between reactive mesothelial cells and poorly differentiated malignant cells.[10] Earlier methods of CB preparations did not receive much attention, probably due to the lack of standardized technique. In fact the main problem with the CB preparation is the risk of losing material during preparation. Some researchers used agar, plasma/thromboplastin to bind the sedimented cells, but they have some disadvantages.[7,8]

The advantages of the CB procedure include:

1. Recognition of histological patterns of diseases that sometimes cannot be identified reliably in conventional smears.
2. Possible to study multiple sections by routine staining, special staining and immunocytological procedures.
3. Less cellular dispersal, which permits easier microscopic observation than do traditional smears.
4. Less difficulty in spite of background showing excess blood on microscopic observation.
5. Possibility of storing slides for retrospective studies. Storage of the CS is a practical problem.[11]

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For this reason, an attempt was made to prepare and analyze both CS and CB from the same specimen. In this study, due consideration was given to age, sex, site of effusion, clinical and radiological findings, to arrive at a final diagnosis and also to identify the primary malignant lesion. Cell blocks may provide diagnostic information complementary or additional to that obtained from an examination of the cell smears. However, morphological preservation is often unsatisfactory in cell blocks processed by routine schedules used for surgical specimens. The 7.5% buffered formalin is the optimal formalin solution and a shortened time through xylene is desirable. Nevertheless, xylene has a marked shrinking effect on cells. If the usefulness of a cell block is to be maximized, fixation and processing of the samples has to be modified so that the morphology approaches that of the conventional paraffin sections of the surgical specimens.[7] In our study, we used 10% alcohol–formalin as a fixative for the CB preparation. By doing this, we got better cellularity as formalin minimized the cell loss by forming protein cross links and gel formation that could not be dissolved by various chemicals used for processing.

Apart from increased cellularity, better morphological details were also obtained with CB, which included, preservation of the architectural patterns such as, cell balls, papillae and three-dimensional clusters, better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin details [Figure 3]. All these features increased the sensitivity of the diagnosis of malignancies by the CB methods, which were reported as negative in CS.

Reactive mesothelial cells have in the past been responsible for simulating malignancy in CS, largely due to the formation of rosettes, pseudoacini, or acini, with or without the presence of prominent nucleoli. The CB effectively puts both the features in their proper prospective: That is, the nucleoli do not appear as prominent as in the CS, and the pseudoacinar or acinar structures can be better appreciated when present, in the CB. Similar findings were noticed in the Dekker and Bupp study.[3] More important still, this 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 is seen in the CB, together with mucin, when stained for mucin, and are indicative of malignancy. The other advantage of CB is concentration of cellular material in one small area that can be evaluated at a glance with all cells lying in the same focal plane of the microscope. It bridges the gap between cytology and histology.[3]

Comparison of the cytodiagnosis of malignant effusions in the present study been compared with other studies. An

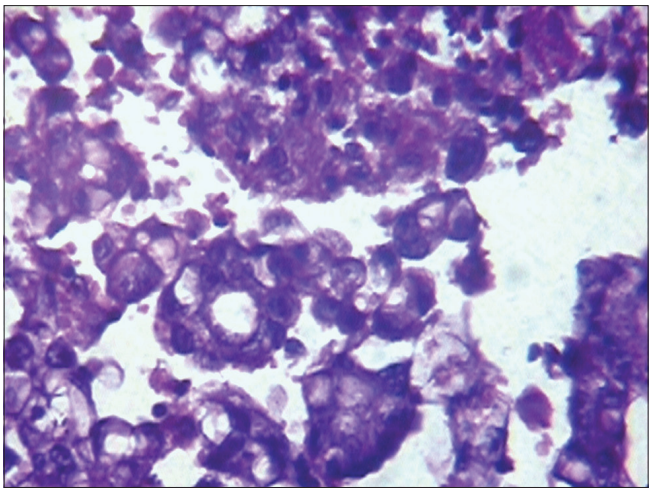

Figure 3: Photomicrograph showing acini, papillae and pleomorphic cells in CB (H and E, ×400)

additional yield for malignancy is similar to the results of the Dekkar and Bupp[3] study. They reported that samples obtained by the combined CB and CS techniques for malignant lesions were double that of the CS technique only.

The present study results for primary lesions were correlating with the Sears and Hajdu[4] and Johnston[12] studies.Sears and Hajdu[4] reported that the most common primary neoplasms causing pleural effusions were carcinoma of the breast (24%), followed by lung (19%) and lymphoreticular system (16%), and in 15% of the cases the primary site was unknown. In our study for pleural fluid analysis, carcinoma of the breast (30%) was the most common primary followed by primary in the lung (20%) and gastrointestinal tract (20%) and in 30% of the cases the primary site was unknown. Most of the tumors were of the adenocarcinoma type. In the present study, out of seven unknown primary cases, one case was suspected to be from papillary carcinoma of the thyroid, as the morphological features showed the presence of papillae, optically clear nuclei and psammoma bodies. In another case carcinoid tumor was suspected due to the presence of morphological features and clinical manifestations such as headache and flushing. The 5-HT assay was advised, but the patient was lost to follow-up. In another case, the radiological features revealed findings of pancreatic carcinoma, but this case was also lost to follow-up. In these three suspected cases, retrospective clinical examination and other investigations did not show primary in the lung, breast, gastrointestinal tract, or genitourinary sites. In the remaining four unknown cases, the primary was not known, as the clinical details were not available and these patients also were lost to follow up.

We noted the presence of pericellular lacunae in more than 60% of the cases of adenocarcinoma, especially of the mucin secreting type, characterized by large cell clusters.[6] Bull's

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eye (Target) inclusion - like findings were seen in one case of metastatic adenocarcinoma of the gastrointestinal tract, which was an additional finding in the diagnosis of malignant effusions.[13] Hence, the sections from CB provided additional information for a definite diagnosis, as it allowed recovery of minute cellular material and was valuable for histochemical and immunohistochemical methods.[14,15]

To conclude, our present study results showed that the cellblock technique, by using 10% alcohol–formalin as a fixative, was a simple, inexpensive method, and did not require any special training or instrument. The CB method yielded more cellularity and better architectural patterns which improved the diagnosis of malignancy by 15%. Multiple sections could be obtained if required for special stain or an Immunohistochemistry (IHC) study. Therefore, the CB technique could be considered as a useful adjuvant in evaluating fluid cytology for a final cytodiagnosis, along with the routine CS method.[16]

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